Sordarin – the antifungal antibiotic with unique *modus operandi*

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Abstract: Fungal infections cause serious problems in many aspects of human life; especially infections by fungal species represent problems in immunocompromised patients. Current antifungal antibiotics target various metabolic pathways, predominantly the cell wall or cellular membrane. However, numerous compounds are available to combat fungal infections, their efficacy is far from being satisfactory and some of them display substantial toxicity. The emerging resistance represents a serious issue as well; thus, there is a considerable need for new anti-fungal compounds with lower toxicity and higher effectiveness. One of the unique antifungal antibiotics is sordarin, the only known compound that acts on the fungal translational machinery *per se*. It has been shown that sordarin inhibits protein synthesis at the elongation step of the translational cycle, acting on eukaryotic elongation-factor-2. In this review, we are aiming to deliver a robust scientific platform promoting the development of antifungal compounds, especially focusing on molecular action of sordarin.

Keywords: sordarin, ribosome, translation, translocation, eukaryotic elongation factor 2 (eEF2), translational GTPase

Abbreviation: fingolimod (FTY720), siderophore iron transporter (Sit1), elongation factor 2 (eEF2), histone deacetylase 2 (Hos 2), bromodomain and extra-terminal (BET), 3-phosphoinositide-dependent protein kinase 1 (Pdk1), high osmolarity glycerol (HOG), reactive oxygen species (ROS), invasive fungal infections (IFIs), half-maximal inhibitory concentration (IC_{50}), tetrahydropyran (THP), concentrations of compounds required to achieve 50% inhibition (Tox_{50}), pharmacokinetic parameters (PK), area under the concentration-time curve (AUC), maximum concentration of drug in serum (C_{max}), pharmacodynamics (PD), time that serum drug concentrations remain above the MIC (t > MIC), area under the survival time curve (AUSTC), fusidic acid (FA), sordarin-specificity region (SSR), cryo-electron microscopy (cryo-EM), GTPase-associated center (GAC), sarcin-ricin loop (SRL)

1 Introduction

It is estimated that there are 2.2-3.8 million fungal species on earth (Hawksworth & Lucking, 2017), and fungal infections represent a serious concern in agriculture and human health. Pathogenic fungi are frequently called hidden killers (Brown, Denning, Gow, Levitz, Netea & White, 2012) and approximately 1.5 million people lose their lives worldwide annually due to invasive mycoses (Kupferschmidt, 2019), while over 1 billion are exposed and affected (Bongomin, Gago, Oladele & Denning, 2017). Among them, *Candida*, *Cryptococcus*, and *Aspergillus* species pose the most serious threats affecting more than 1 million people every year (Janbon, Quintin, Lanternier &

d'Enfert, 2019). Especially *Candida albicans*, widely distributed in nature, accounts for 70%-80% of candidiasis cases (Chin, Lee, Rusliza & Chong, 2016) causing an approx. 50% mortality rate in immunocompromised patients with life-threatening systemic and bloodstream infections (Bongomin, Gago, Oladele & Denning, 2017). It should be underlined that fungal infections are difficult to diagnose and the available therapeutics are currently not highly effective (Kupferschmidt, 2019). Thus, the discovery and/or development of antifungal agents against e.g. *Candida albicans* fungal infections represent a huge challenge.

So far, vast number of strategies/targets based on antifungal compounds targeting diverse biological pathways have been developed to combat fungal infections (Figure 1). However, only some of them are widely used to treat fungal infections. The classic therapies include application of polyenes, flucytosine, azoles, and echinocandins (Campoy & Adrio, 2017; Perfect, 2017); except for flucytosine, which acts on DNA synthesis, they mainly target the cell wall and membrane metabolism, including ergosterol biosynthesis (Zida, Bamba, Yacouba, Ouedraogo-Traore & Guiguemde, 2017). The therapeutic compounds are represented by polyenes (amphotericin B (Bezerra, Silva, Santos-Veloso, Lima, Chaves-Markman & Juca, 2020; Liu, Chen & Yang, 2017), nystatin (Khalandi et al., 2020), natamycin (Guo, Karimi, Fu, G & Zhang, 2020)); azoles (imidazoles: clotrimazole (Grimling, Karolewicz, Nawrot, Wlodarczyk & Gorniak, 2020), miconazole (Xu et al., 2020), ketoconazole (Lou et al., 2019); triazoles: fluconazole (Khalandi et al., 2020), itraconazole (Lou et al., 2019), voriconazole (Lou et al., 2019), posaconazole (Chen, Krekels, Verweij, Buil, Knibbe & Bruggemann, 2020), efinaconazole (Noguchi et al., 2018), isavuconazole (Ellsworth & Ostrosky-Zeichner, 2020)); allylamines (terbinafine (Kastamonuluoglu, Buyukguzel & Buyukguzel, 2020)), morpholines (amorolfine (Ghannoum, Long, Kunze, Sarkany & Osman-Ponchet, 2019)) and thiocarbamates (tolnaftate (Emam, Abdelrahman, Abdelaleem & Ali, 2019)). Additionally, the β-glucan synthetase pathway (Zida, Bamba, Yacouba, Ouedraogo-Traore & Guiguemde, 2017) is targeted by echinocandin (caspofungin (Lee et al., 2018), micafungin (Wasmann, Muilwijk, Burger, Verweij, Knibbe & Bruggemann, 2018), anidulafungin (Cushion et al., 2018)) and ibrexafungerp (SCY-078) (Larkin et al., 2017). Moreover, chitin synthesis is inhibited by nikkomycins (Larwood, 2020) and polyoxins (Osada, 2019). Additionally, a promising target towards the cell wall and membrane is the glycosylphosphatidylinositol (GPI anchor) synthesis pathway affected by gepinacin (Liston et al., 2020) and APX001 (Wiederhold et al., 2019). Additionally, it has been reported that bifunctional small molecules (Cloudbreak molecules) may efficiently suppress fungal growth by acting effectively on the cell wall (Jones et al., 2019). Also, sphingolipid synthesis is blocked by fingolimod (FTY720) (Podbielska, Krotkiewski & Hogan, 2012) and aureobasidin A (Munusamy, Vadivelu & Tay, 2018). Furthermore, amino acid transporters can be considered as promising

targets for antifungals like sinefungin (McCarthy & Walsh, 2018). Also, the siderophore iron transporter (Sit1) can be targeted by ASP2397 (VL-2397) (Dietl et al., 2019). In terms of translation, isoleucyl-tRNA synthetase is targeted by icofungipen and cispentacin (McCarthy & Walsh, 2018), and leucyl-tRNA synthetase is targeted by tavaborole (McCarthy & Walsh, 2018; Sharma & Sharma, 2015). The translational machinery, especially elongation factor 2 (eEF2), is targeted by sordarin (McCarthy & Walsh, 2018), and melleolides have recently been found to affect eEF2 as well (Dorfer et al., 2019). The transcription can also be considered as a good target, with the DNA and RNA synthesis pathways inhibited by pyrimidine analogs (Aryan, Beyzaei, Nojavan, Pirani, Samareh Delarami & Sanchooli, 2019), flucytosine (Nivoix, Ledoux & Herbrecht, 2020) or yatakemycin (Igarashi et al., 2003). Also, the newly discovered MGCD290 targets histone deacetylase 2 (Hos2) (Pfaller, Rhomberg, Messer & Castanheira, 2015), and bromodomain and extra-terminal (BET) family proteins are targeted by dibenzothiazepinone (Mietton et al., 2017). Additionally, the microtubule biosynthesis pathway represents a target for griseofulvin (Kartsev et al., 2019) and vinblastine (Kopecka & Gabriel, 2009). Furthermore, general metabolism pathways are also targeted by several biochemicals, e.g. the glyoxylate cycle (Bae et al., 2015), trehalose pathway (Miao et al., 2017), and aspartate synthesis pathway (Bareich, Nazi & Wright, 2003). Last but not least, the signal transduction pathway and stress response system are also considered as targets for antifungals. The RAS pathway can be blocked by farnesylation and prenylation inhibitors (Perfect, 2017), fungal 3-phosphoinositide-dependent protein kinase 1 (Pdk1) is inhibited by KP-372-1 (Baxter, DiDone, Ogu, Schor & Krysan, 2011), the high osmolarity glycerol (HOG) pathway is inhibited by fludioxonil (Randhawa, Kundu, Sharma, Prasad & Mondal, 2019) and ambruticins (Vetcher, Menzella, Kudo, Motoyama & Katz, 2007), reactive oxygen species (ROS) and oxidative damage are linked with citronellal (Saibabu, Singh, Ansari, Fatima & Hameed, 2017), and the calcineurin pathway can be affected by tacrolimus (Jung & Yoon, 2020) and cyclosporine (Liao & Sun, 2018) (Figure 1).

Interestingly, the fungal protein synthesis pathway is not frequently targeted, as in the case of bacteria, where approx. 50% of anti-bacterial antibiotics act on the transitional machinery. Besides inhibitors of tRNA synthetases (McCarthy & Walsh, 2018), sordarins are the only class of compounds that have been reported to be used as antifungal agents acting on the translational machinery, so far. Thus, it can be concluded that many metabolic pathways are targeted by a number of compounds that can be regarded as specific antifungals; however, one of the most critical metabolic cycles, i.e. protein synthesis, is affected by only one compound, sordarin, which has extraordinary specificity. Sordarins were perceived as one of the most promising antifungal agents to fight invasive fungal infections (IFIs). There has been significant development of a vast number of sordarin derivatives displaying extraordinary *in vitro* and *in vivo* efficacy

with high specificity toward numerous fungal species and very low toxicity which makes sordarins much safer than the drugs applied nowadays. Importantly, sordarins display a unique *modus operandi* targeting the fungal translational machinery exclusively, leaving the human or other organisms' translational systems unaffected. Despite many studies, its actions remain to be thoroughly described. This review is focused on providing the newest and comprehensive insight into the mechanism of sordarin action and highlighting new perspectives on the way to develop effective antifungal agents.

2 Sordarin – *modus operandi*

2.1 Chemical structure

Sordarin ($C_{27}H_{40}O_8$) was first isolated from *Sordaria araneosa* S2266 (*Sordariaceae*) in the 1960s (Hauser & Sigg, 1971) and patented in 1969 under the name SL-2266 (Sigg & Stoll, 1969). It is a tetracyclic diterpene glycoside composed of diverse glycones which can be replaced by additional moieties, and a unique 5/6/5/5 fused tetracyclic ring system as the core element with 4 groups (Figure 2): a glycone group (Figure 2, R1), an isopropyl group (Wu & Dockendorff, 2019) (Figure 2, R2), an essential carboxylic acid group (Figure 2, R3), and a formyl group which can be optionally replaced by nitrile (Cuevas, Lavandera & Martos, 1999; Liang, Schule, Vors & Ciufolini, 2007; Wu & Dockendorff, 2019) (Figure 2, R4). All groups are in a vicinal arrangement with a high dihedral angle to avoid internal hemiacetalization (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998); the last one, i.e. a methyl group, is placed in a five-membered ring (Figure 2, R5) (Wu & Dockendorff, 2019). Due to the unique common tetracyclic diterpene core with a norbornene system, all known sordarin analogs display antifungal activity (Liang, 2008). Sordarins were isolated from various natural sources (Table 1). For example, sordarin with such special moieties as in SCH57404 isolated from an unidentified fungus SCF1082A has a rare sordaricin skeleton and a tricyclic sugar moiety (Coval, Puar, Phife, Terracciano & Patel, 1995). Xylarin a, b, c, first isolated from culture fluids of a wood-inhabiting *Xylaria* species, contains a tricyclic uronic acid moiety (Schneider, Anke & Sterner, 1995). Trichosordarin A isolated from *Trichoderma harzianum* R5 has a specific norditerpene aglycone reported to be the only sordarin analog that is toxic to the marine zooplankton *Artemia salina* (Liang, Ma & Ji, 2020). Moriniafungin containing a 2-hydroxysebacic acid residue linked to C-30 of the sordarose residue of sordarin through a 1,3-dioxolan-4-one ring was isolated from *Morinia pestalozzioides* (Basilio et al., 2006) and *Setosphaeria rostrata* F3736 (Park, Park, Kim, Lee & Kim, 2020). Additionally, TA26-15 was found in *Curvularia hawaiiensis* from the South China Sea together with 6 additional homologs, moriniafungins B-G (Zhang et al., 2019) (Table 1). The class of

naturally occurring sordarin antibiotics was significantly enlarged by the chemical synthesis approach (Chiba, Kitamura & Narasaka, 2006; Liang, 2008; Schule, Liang, Vors & Ciufolini, 2009). It includes 3-O-substituted derivatives (Arribas et al., 2002), 3',4'-fused dioxolane and dioxane derivatives (Bueno, Cuevas, Fiandor, Garcia-Ochoa & Gomez de las Heras, 2002), core-modified derivatives (Regueiro-Ren et al., 2002), 2',3'-fused oxirane derivatives (Castro, Cuevas, Fiandor, Fraile, de las Heras & Ruiz, 2002), and 3',4'-fused alkyl-tetrahydrofuran derivatives (Bueno, Chicharro, Fiandor, Gomez de las Heras & Huss, 2002) or modification of alkylthio, morpholinyl, alkanesulfonate, oxazepane, or trisubstituted tetrahydrofuran (Hanadate et al., 2009; Kaneko, Arai, Uchida, Harasaki, Fukuoka & Konosu, 2002; Serrano-Wu, Du, Balasubramanian & Laurent, 2002), an alkyl-modified side-chain with *n*-nonyl, *n*-octyl, *n*-heptyl, *n*-hexyl, *i*-pentyl, *n*-pentyl, *i*-Bu, *n*-Bu, n-Pr, Et, and Me (Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998) (**Błąd! Nie można odnaleźć źródła odwołania.**). Additionally, the group of sordarins has been enlarged by azasordarin derivatives, including sordarin oxime derivatives (Figure 3, A) (Serrano-Wu et al., 2002b), sordarin morpholino derivatives (Figure 3, B) (Serrano-Wu et al., 2003), *N*-substitued 1,4-oxazepanyl sordarins (Figure 3, C) (Kaneko, Arai, Uchida, Harasaki, Fukuoka & Konosu, 2002), oxazepine sordarins (Figure 3, D) (Serrano-Wu et al., 2002a), isoxazoline sordarins (Figure 3, E) (Serrano-Wu et al., 2002b), isoxazole sordarins (Figure 3, F) (Serrano-Wu et al., 2002b), FR29581 (Figure 3, G) (Hanadate et al., 2009), and GM258383 (Figure 3, H) (Dominguez & Martin, 2001). These derivatives were mainly centered on the glycoside part to improve the antifungal spectrum, cell uptake, and biological activity or to reduce toxicity (Table 2). Also, their stability represents an important issue, because such sordarins like sordarose or sordaricin are easily decomposed by cytochrome P-450-mediated hydrolytic cleavage at cyclopentane C-6 and C-7 positions in serum and liver (Cuevas, Lavandera & Martos, 1999; Hauser & Sigg, 1971). The effect of the chemical modifications can be shown by an example where replacement of the sugar moiety (Figure 2, R1) with a short alkyl chain changed the half-maximal inhibitory concentration (IC_{50}) of sordarin toward *S. cerevisiae* from 10 μg/ml to 0.00001 μg/ml (Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998). Replacement of -CHO with -CN (Figure 2, R4) increased the sordaricin IC_{50} to 20μg/ml, while the original one displayed very low activity (Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998). Additionally, reducing the tetracyclic skeleton to the cyclopentane ring and replacement of tetrahydropyran (THP) at the hydroxyl position improved lipophilicity (Wu & Dockendorff, 2018) and resulted in an over 6-fold increase in the minimal inhibitory concentrations (MIC) (Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998). Thus, there is large room for sordarin improvement, making this compound still not fully explored from the chemical and biological point of view.

2.2 Biological properties

2.2.1 *In vitro* activity

Sordarins exhibit potent antifungal activity *in vitro* against many life-threatening pathogens, e.g. *Candida albicans* (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Okada et al., 1998; Schneider, Anke & Sterner, 1995), *Pneumocystis carinii*, and *Cryptococcus neoformans* (Basilio et al., 2006; Okada et al., 1998), and against other less common pathogens like *Absidia glauca* (Daferner, Mensch, Anke & Sterner, 1999), *Candida glabrata* (Basilio et al., 2006; Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Okada et al., 1998), *Mucor miehei*, *Nematospora coryli*, *Paecilomyces variotii* (Daferner, Mensch, Anke & Sterner, 1999; Weber, Meffert, Anke & Sterner, 2005), *Saccharomyces cerevisiae* (Basilio et al., 2006; Daferner, Mensch, Anke & Sterner, 1999; Davoli, Engel, Werle, Sterner & Anke, 2002; Okada et al., 1998; Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998), *Zygorhynchus moelleri* (Daferner, Mensch, Anke & Sterner, 1999), and many more (Table 2). Importantly, the range of sordarins was expanded over time as new compounds were discovered, including natural sordarin analogs and chemical derivatives (Table 2). The *in vitro* activity of numerous sordarin classes was tested against over 50 species, considering MIC and IC_{50}. Table 2 provides a comprehensive list of sordarin compounds with the range of concentrations affecting fungal species. The presented data are a compilation of available information, because of response differences among strains, within the same species. For example, the MIC of GR135402 is 0.03 μg/ml for *Candida albicans* strain C316, 0.008 μg/ml for strain 2005E, and 0.06 μg/ml for strains 1208E, 2402E, and 2381E (Kinsman et al., 1998). Especially, this is true for numerous clinical isolates, which react differently; for example, GM237354 acts differently toward clinical isolates of *Cryptococcus neoformans* from HIV-infected patients with cryptococcosis from Spain, Argentina, Brazil, and Cuba, and it was found that MIC varied significantly in range from 0.003 to 2.0 μg/ml (Torres-Rodriguez, Morera, Baro, Lopez, Alia & Jimenez, 2002). It should also be mentioned that the variation in sordarin action depends on experimental conditions, which affect *in vitro* analyses. For instance, the MICs of BE-31405 and sordarin toward *Candida albicans* strain IFO1270 at pH 5.4 are in the same range of 3.1 μg/ml; however, at pH 7.0, the MIC value increases to 50 and 100 μg/ml, respectively. A similar situation has been reported for sordarin derivative TIMM3170, i.e. the MIC values against *Candida albicans* were 3.1 and 1.56 μg/ml at pH 4.5 and 25 μg/ml at pH 7.0 (Okada et al., 1998).

Considering particular species, *Candida albicans* representing the biggest threat have been widely studied in connection with the inhibition activity of various sordarin classes. It has been reported that sordarin (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Okada et al., 1998; Schneider, Anke & Sterner,

1995), sordaricin B (Weber, Meffert, Anke & Sterner, 2005; Zhang et al., 2019), BE-31405 (Okada et al., 1998), moriniafungin, moriniafungin B-G (Zhang et al., 2019), FR290581 (Hanadate et al., 2009), R-135853 (Kamai, Kakuta, Shibayama, Fukuoka & Kuwahara, 2005), GM 160575, GM 191519, GM 193663, GM 211676, GM 222712 (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998), GM 237354 (Aviles, Falcoz, San Roman & Gargallo-Viola, 2000; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998), GR135402 (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Kinsman et al., 1998), GW 471552, GW 471558, GW 479821, GW 515716, GW 570009, GW 587270 (Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001; Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001), 7-hydroxysordarin (Hall et al., 2001), 4'-O-demethylsordarin (Hall et al., 2001), 2'-O-acetylsordarin (Hall et al., 2001), 7-hydroxy-4-O-demethylsordarin (Hall et al., 2001), and other derivatives display activity toward *Candida albicans* (Table 2). It should be pointed out that many other *Candida* species are affected by various sordarins, e.g. *Candida glabrata* (Serrano-Wu et al., 2003) (Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001) (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998), *Candida kefyr* (*Kluyveromyces marxianus*) (Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998), *Candida krusei* (*Pichia kudriavzevii*) (Basilio et al., 2006), *Candida neoformans* (Hanadate et al., 2009), *Candida parapsilosis* (Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001), *Candida pseudotropicalis* (Kinsman et al., 1998), and *Candida tropicalis* (Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998) (Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001; Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001) (Table 2). Besides *Candida* species, many other yeast and yeast-like fungi are affected by various sordarin derivatives; especially *Saccharomyces cerevisiae*, so-called baker yeast, has been widely used as an experimental model to test the activity of sordarins (Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998). *S. cerevisiae* are efficiently inhibited by various sordarin derivatives with a MIC range of 1.56-50 µg/ml (Basilio et al., 2006; Daferner, Mensch, Anke & Sterner, 1999; Davoli, Engel, Werle, Sterner & Anke, 2002; Okada et al., 1998; Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998). Others yeast like *Nematospora coryli* also display high sensitivity toward sordarin (Basilio et al., 2006; Daferner, Mensch, Anke & Sterner, 1999; Davoli, Engel, Werle, Sterner & Anke, 2002; Okada et al., 1998; Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998). Similarly, *Blastoschizomyces capitatus*(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001), *Geotrichum clavatum* (Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-

Viola, 2001), and *Trichosporon beigelii* (Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998) species have high sensitivity toward numerous sordarins. Additionally, *Cryptococcus neoformans*, which is the major human and animal pathogen, displays high sensitivity toward numerous sordarin derivatives, such as GM 191519 with IC_{50} 0.005 μg/ml (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998) and GM 237354 with MIC 0.015-0.25 μg/ml (Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998).

Importantly, filamentous fungi, which form a large class of pathogens, display significant sensitivity toward various sordarins. The growth of *Aspergillus fumigatus* and *Aspergillus flavus* is effectively inhibited by GM 222712 (Table 2) (Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998). Additionally, other fungi are affected by numerous sordarins, e.g. *Absidia glauca* (Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998) (Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001), *Cladosporium cladosporioides* (Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998), *Fusarium oxysporum* (Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001), *Mucor miehei*, *Paecilomyces variotii*, *Penicillium islandicum*, *Penicillium notatum*, and *Zygorhynchus moelleri* (Daferner, Mensch, Anke & Sterner, 1999). Also, *Ustilago nuda* is inhibited by xylarin (Schneider, Anke & Sterner, 1995; Weber, Meffert, Anke & Sterner, 2005). Additionally, other fungal species display sensitivity toward sordarins, including zygomycetes *Absidia corymbifera* and *Cunninghamella bertholletiae* and dermatophytes *Epidermophyton floccosum*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. In general, a majority of species that belong to the Fungi kingdom are sensitive toward various sordarins, which makes this compound a very promising but underestimated antibiotic.

Sordarin has also been tested *in vitro* against bacterial species and mammalian cells. In the mammalian experimental model, sordarin and its various derivatives showed a slight toxic/inhibitory effect. Using rabbit reticulocytes as target cells, sordarin as well as GM160575, GM191519, GM193663, GM211676, GR135402 (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998), and BE-31405 (Okada et al., 1998) were tested and the IC_{50} were over 100 μg/ml, which indicated that sordarin is not toxic to eukaryotes. Additionally, using several cell lines, i.e. HL-60, L12102, HeLa, COS-7, Colo-320, and HepG2, the toxicity of sordarin derivatives were tested, and obtained IC_{50} was in the range of 50-100 μg/ml and above, once again showing little toxicity toward mammalian cells (Daferner, Mensch, Anke & Sterner, 1999). In other experimental models, i.e. cell lines MDCK, MRC-5, and MH1C1 used to evaluate toxicity of GW471552, GW471558, GW479821, GW515716, GW570009, and GW587270, the sordarins showed little toxicity (Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001). Additionally, sordarins, including sordarin B,

hydroxysordarin, sordaricin, and other derivatives, were tested against bacteria *Bacillus brevis*, *B. subtilis*, *Enterobaccter dissolvens*, and *Sarcina lutea* with all the results of MIC >50 μg/ml, indicating that there was no inhibition of bacterial cells (Weber, Meffert, Anke & Sterner, 2005).

Importantly, there are no reports on sordarin resistance in naturally isolated fungi. An *in vitro* analysis using GW471558 and four *Candida albicans* isolates showed that with increasing concentrations of GW471558 in the medium, the rate of resistance gain was very low, compared to other anti-fungal compounds (Odds, 2001). Thus, sordarin can be considered as a very good antifungal toward resistant strains. For example, in the case of the fluconazole-resistant *Candida albicans*, the MIC values were 16-128 μg/ml for fluconazole, 0.03-0.12 μg/ml for itraconazole, and 0.12-0.25 μg/ml for amphotericin B. In turn, the MIC values for sordarin derivatives GM193633, GM 211676, GM 222712, GM 237354, GW 479821 (Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998), GW471552, GW 471558, GW515716, GW 570009, and GW 587270 were lower than 0.06 μg/ml, which indicated a superior inhibitory effect (Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001).

In summary, sordarin display extraordinary specificity and efficacy toward all organisms from the Fungi kingdom, contrary to other species that are not affected. Importantly, various sordarin derivatives efficiently act *in vitro* on many fungi that cause human infections, underscoring the fact that these compounds represent unique chemicals with promising properties as antibiotics.

2.2.2 *In vivo* activity

Sordarins act efficiently against various fungal species *in vitro* (Table 2), and further *in vivo* analyses confirmed their high effectiveness toward fungal infections. Several sordarin derivatives were analyzed, including GM211676(Clemons & Stevens, 2000), GM193663, GM222712 (Aviles, Pateman, San Roman, Guillen, Gomez De Las Heras & Gargallo-Viola, 2001; Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000), GM237354 (Aviles, Falcoz, Guillen, San Roman, Gomez De Las Heras & Gargallo-Viola, 2001; Aviles, Falcoz, San Roman & Gargallo-Viola, 2000; Martinez, Regadera, Jimenez, Santos & Gargallo-Viola, 2001), GM191519, GM219771 (Aviles et al., 2000), GW471552, GW471558 (Jimenez, Martinez, Aliouat el, Caballero, Dei-Cas & Gargallo-Viola, 2002; Martinez et al., 2001), GW 531920 (Odds, 2001), GR 135402 (Kinsman et al., 1998), R-135853 (Kamai, Kakuta, Shibayama, Fukuoka & Kuwahara, 2005), FR290581 (Hanadate et al., 2009), azasordarin, and azasordarin derivates 7a, 7b (Serrano-Wu et al., 2003). The analyses were performed with such model organisms as monkeys, rats, mice, rabbits (Aviles, Pateman, San Roman, Guillen, Gomez De Las Heras & Gargallo-Viola, 2001), and dogs (Gargallo-Viola, 1999; Odds,

2001). The *in vivo* evaluation of the efficiency of sordarins was focused on several pathogens, i.e. *Candida albicans* (Aviles, Falcoz, San Roman & Gargallo-Viola, 2000), *Pneumocystis carinii* (Aviles et al., 2000), *Aspergillus fumigatus* (Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000), *Histoplasma capsulatum* (Graybill, Najvar, Fothergill, Bocanegra & de las Heras, 1999), and *Coccidioides immitis* (Clemons & Stevens, 2000; Deresinski, 2001). The best-studied animal model was mice exposed to *Candida albicans* infections. Sordarin GR135402 was tested in mice with systemic candidiasis treated with increasing amounts of the compound from 1.56 to 100 mg/kg. It contributed to a high survival rate of the infected animals and, importantly, there was no significant toxicity observed in the uninfected animals, indicating that GR135402 displayed high drug safety (Kinsman et al., 1998). Also, the activity of sordarin analogues toward candidiasis were studied in other animal models treated with various doses orally and intravenously, indicating that sordarins were very effective and displayed low toxicity (Table 3).

Comprehensive *in vivo* analyses were conducted with sordarin GM237354~~,~~ which showed extraordinary *in vitro* efficiency (Table 2) (Martinez, Regadera, Jimenez, Santos & Gargallo-Viola, 2001). In a murine model, numerous pharmacokinetic parameters (PK) were analyzed, including the area under the concentration-time curve (AUC), maximum concentration of drug in serum (C_{max}), and pharmacodynamic (PD) parameters, i.e., the time that serum drug concentrations remain above the MIC ($t >$ MIC). Also, treatment efficacies were evaluated in terms of the area under the survival time curve (AUSTC) and kidney fungal burden (log·CFU/gram). The mice were challenged intravenously with *Candida albicans*, and all analyses showed high therapeutic efficacy of GM237354 at different dosing regimens; especially, the AUC value at which 50% of the maximum effect was reached (AUC_{50}) were 21.7 and 34.7 mg · h/ml for 8 and 4 h intervals, with reduction in kidney burden (Aviles, Falcoz, San Roman & Gargallo-Viola, 2000). Additionally, the therapeutic effect of GM237354 was investigated in an experimental system with oral delivery of *Candida albicans* using immunosuppressed rats as an infection model. The histopathology and morphometry studies showed that the percentage of epithelium occupied by *C. albicans* hyphae in animals treated with as little as 7.5 mg/kg/day was significantly decreased, indicating that the sordarin derivative was highly effective against candidiasis in orally infected immunosuppressed rats. GM237354 was also studied in in terms of correlations between sordarin pharmacokinetic properties and therapeutic efficacy. It was showed that to reach efficacy in the range of 90% survival, the AUC was predicted as 67 μg·h/ml (Aviles, Falcoz, San Roman & Gargallo-Viola, 2000). Moreover, the activity of GM237354 has *in vitro* - *in vivo* correlations, suggesting coherent action of sordarin in respect to *C. albicans* infection in mice experimental model (Aviles, Falcoz, Guillen, San Roman, Gomez De Las Heras & Gargallo-Viola, 2001). However, the evaluation

of efficiency can be affected by the experimental model organism used. For example, the C_{max} value for rabbit and monkey was 2-fold higher than that in mouse or rat. In monkey, the largest AUC of 161 μg·h/ml, the longest $t_{1/2}$ of 1.73 h, and the lowest Cl_p of 2.1 ml/min/kg were determined. The C_{max} parameter was similar between rabbit and rat, while AUC in mouse was as small as 17.8 μg·h/ml and Cl_p was higher, i.e. 19 ml/min/kg (Aviles, Pateman, San Roman, Guillen, Gomez De Las Heras & Gargallo-Viola, 2001). Besides, compared to sordarin FR290581, sordarin GM237354 showed 100 times higher activity in mouse serum, 50 times higher C_{max}, and 10 times longer half-life of 3.4h *in vivo* at the dose of 2 mg/kg (Hanadate et al., 2009). Compared to fluconazole, lower kidney burden was detected at the dose of 20 mg/kg (Hanadate et al., 2009). Furthermore, another sordarin R-135853 exhibited good dose-dependent efficacy in an experimental murine model with hematogenous candidiasis upon subcutaneous and oral therapy. Importantly, R-135853 had a high level of oral bioavailability with 63% of absorption at 20 mg/kg, but the half-life was as short as 1.1 and 0.47 h after administration of 20 mg/kg orally and 2 mg/kg intravenously, respectively. Notably, R-135853 eradicated esophageal candidiasis at 10 and 50 mg/kg/ doses, respectively, while fluconazole did not reduce the viable cell counts significantly at the same administration regime (Kamai, Kakuta, Shibayama, Fukuoka & Kuwahara, 2005). Thus, sordarins display extraordinary efficacy toward fungal infections; but the half-life of sordarins, i.e. in the range of 0.3-4 hr, is a concern. However, in the course of study on stability of sordarins, it was shown that chemical modifications may provide a possibility to improve this parameter (Serrano-Wu et al., 2003).

Pneumocystosis is considered a serious lung infection caused by opportunistic pathogen *Pneumocystis carinii* in immunocompromised patients (Aviles et al., 2000). It has been shown that sordarins GM191519, GM237354, GM193663, and GM219771, which have high effectiveness *in vitro* (Table 2), also display a similar correlation *in vivo* (Table 4) and, what is more, the efficacy of these sordarins are comparable to the commercially available medicines such as pentamidine, atovaquone, and TMP-SMX (Aviles et al., 2000). In a rat pneumocystosis models, over 90% reduction of *Pneumocystis carinii* cysts in lungs was reported by 5 mg/kg of GW471552, GW471558 (Jimenez, Martinez, Aliouat el, Caballero, Dei-Cas & Gargallo-Viola, 2002), GM237354, and GM 193663 (Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000)~~,~~ which is comparable with the septrin/cotrimoxazole antibiotic frequently used to cure pneumocystosis (Table 4). Importantly, comparison of septrin/cotrimoxazole with GW471552 and GW471558, the sordarins showed higher activity and lower cysts survival in the lung of infected rats, although GW471558 had to be administered at a higher dose than GW471552 (Jimenez, Martinez, Aliouat el, Caballero, Dei-Cas & Gargallo-Viola, 2002). In several studies on rat models (Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000), it has been proposed that 1 mg/kg

of GM237354 represents the optimal dose for several other sordarins which indicates that sordarins display much higher effectiveness than septrin or cotrimoxazole.

Additionally, infections caused by *Aspergillus fumigatus*, i.e. a pathogenic microorganism posing a serious health threat, were also evaluated in an *in vivo* murine model in the light of GM237354 treatment. The dose used ranged from 10 to 40 mg/kg and was administered subcutaneously every 8 h for 5 days; the treatment significantly reduced the infection, concurrently increasing the survival rate (Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000). Also, a murine model was used to analyze the influence of sordarins on infection caused by *Histoplasma capsulatum*. The infected mice were treated with GM211676A, GM237354A, or GM193663A (Graybill, Najvar, Fothergill, Bocanegra & de las Heras, 1999). GM193663A was the most effective compound and prolonged the survival of the infected mice at a dose of approx. 5 mg/kg/day administered from 9.5 days to over 25 days, indicating that GM193663A had good *in vivo* efficacy in inhibition of severe *Histoplasma capsulatum* infection. Additional important information was provided by analyses of a mice model with systemic coccidioidomycosis. The infected animals were treated with several sordarins: GM193663, GM211676, and GM237354; these derivatives reduced the *Coccidioides immitis* infection in a dose-dependent manner, and GM237354 turned out to be a superior compound; however, a relatively high dose of 100mg/kg/day was required (Clemons & Stevens, 2000).

In summary, the majority of sordarins that have been tested *in vivo* showed extraordinary efficacy toward numerous infections caused by fungal species, having at the same time low toxicity. Thus, the effective clearance of fungal invasions indicates that these compounds represent comparable or even superior antibiotic properties to already known compounds used to combat fungal infections. Nevertheless, the half-life of the tested sordarins represents a serious issue.

3 Biochemistry of sordarin

Sordarin belongs to a class of inhibitors that target the eukaryotic translation cycle, especially the translation elongation step. It should be underlined that the translational machinery represents one of the major targets for antibiotics, especially considering bacterial protein synthesis. This process is subjected to inhibition by vast number of compounds affecting all steps of proteins synthesis, primarily including initiation and elongation (Arenz & Wilson, 2016), and such antibiotics are most widely used to combat bacterial infections (Hutchings, Truman & Wilkinson, 2019). Also, the eukaryotic translational machinery represents a target for numerous inhibitory compounds acting on all major steps of the translational cycle and some of them are regarded as promising therapeutics against a wide range of infectious diseases, cancers, and genetic disorders (Penzo, Montanaro, Trere & Derenzini, 2019; Tahmasebi,

Khoutorsky, Mathews & Sonenberg, 2018). However, sordarin displays the most unique biological feature among known antibiotics acting on eukaryotic cells as it is the only antibiotic that specifically acts on the fungal translational machinery without affecting other eukaryotes.

3.1 Sordarin binding site - eukaryotic elongation factor 2

Sordarins represent the only know antifungal antibiotic acting on the eukaryotic translational machinery exclusively (Capa, Mendoza, Lavandera, Gomez de las Heras & Garcia-Bustos, 1998). The main directly affected element identified so far is the eukaryotic elongation factor 2 eEF2 involved in translation as a factor promoting the translocation of the ribosome during the elongation step of the translational cycle (Dominguez & Martin, 1998; Justice et al., 1998; Liljas & al-Karadaghi, 1997). Importantly, sordarins display exceptional specificity being able to affect fungal eEF2 exclusively; thus, they specifically inhibit the fungal translational system leaving other eukaryotic species, e.g. mammalian, unaffected (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Justice et al., 1998).

Early analyses carried out with the genetic screen approach have shown that a majority of mutations conferring resistance to sordarin are accumulated within eEF2 (Table 5). Sordarin binds specifically to the fungal eEF2-ribosome complex and blocks protein synthesis acting in a similar way to fusidic acid (FA) which blocks bacterial protein synthesis acting on EF-G, a homolog of eEF2 (Gomez-Lorenzo & Garcia-Bustos, 1998; Justice et al., 1998). It was initially reported that sordarin increased the half-life ($t_{1/2}$) of the GDP-eEF2-ribosome complex from less than 0.5 min to approximately 6 min, similarly to FA which increases $t_{1/2}$ up to 10 min (Justice et al., 1998). Noteworthy, it has been shown that, unlike FA, the eEF2-dependent GTP hydrolysis inhibition by sordarin is not dose dependent and kinetic assays have demonstrated an inverted bell-shaped dose-response curve (Dominguez, Gomez-Lorenzo & Martin, 1999). In an uncoupled GTPase activity assay with excess of eEF2 over bulk ribosomes the hydrolyzed GTP decreased consistently presenting a typical dose-dependent inhibition. On the other hand, in a 1:1 molar-ratio of eEF2-ribosomes treated with ricin to obtain structurally/functionally homogeneous ribosomes, the effect was reversed and GTP hydrolysis was stimulated. Thus, it was assumed that ribosomes before the translocation step show high affinity for the eEF-2-GTP complex but low efficiency in stimulating GTP hydrolysis, whereas ribosomes after the translocation step exhibit low affinity for the EF-2-GTP complex but high efficiency in stimulating GTP hydrolysis. Earlier analyses suggested that the high affinity/low catalysis process is inhibited by sordarin while the low affinity/high catalysis process is stimulated by the drug. Accordingly, sordarin is not a direct inhibitor of the GTPase activity since the drug was able to stimulate GTP hydrolysis in certain conditions but blocked protein

synthesis by affecting the eEF2-dependent translocation step (Dominguez, Gomez-Lorenzo & Martin, 1999). The binding site of sordarin to eEF2 has been mapped using numerous approaches. Initially, using the genetic screen and mutagenesis approach, a set of mutants has been identified showing that the binding site for sordarin is located in domain III of eEF2 (Capa, Mendoza, Lavandera, Gomez de las Heras & Garcia-Bustos, 1998; Justice et al., 1998). Initially, the binding site was identified by genetic approaches as a 50-amino-acid segment of the eEF2 protein in the region of 510-567 amino acids and subsequently verified by cross-linking and protease digestion experiments using MS technique (Capa, Mendoza, Lavandera, Gomez de las Heras & Garcia-Bustos, 1998). Further, the binding region was narrowed down by genetic analyses to amino acids 518-524 and defined as a "sordarin-specific region" SSR, displaying a highly conserved set of amino acids for fungal eEF2 such as *S. cerevisiae* or *C. albicans* showing significant differences from the mammalian region at the same time (Figure 4) (Shastry et al., 2001).

3.2 Ribosomal elements conferring sordarin resistance

There are several additional ribosomal elements associated with sordarin resistance, besides eEF2, that represent the primary binding site (Figure 5). The ribosomal protein uL10, previously named as P0 (Ban et al., 2014), was recognized as an element that can be involved in the sordarin action (Justice, Ku, Hsu, Carniol, Schmatz & Nielsen, 1999). The protein belongs to the ribosomal structure called the P-stalk forming a distinct lateral protuberance on the 60S ribosomal subunit (Grela et al., 2012). The P-stalk is formed by the pentameric complex uL10(P1-P2)2 (Grela et al., 2010) with uL10 as an anchoring element of two P1-P2 dimers to the ribosome (Krokowski, Boguszewska, Abramczyk, Liljas, Tchorzewski & Grankowski, 2006). The P-stalk belongs to the GTPase associated center (GAC) which is responsible for interaction with translational GTPases - trGTPases, including eEF2 (Tanzawa et al., 2018) and simulating the GAC dependent GTP hydrolysis by trGTPases (Tchorzewski, 2002). Also, the P-stalk proteins belongs to the ribosomal element allosterically contributing to the decoding event during ribosome action (Wawiorka et al., 2017).

It was first noted that several mutations within the uL10 were related to sordarin resistance; they were located at positions Q139H, W140A, and T144A (Gomez-Lorenzo & Garcia-Bustos, 1998). An additional study showed that the mutations within the *N*-terminal region of the uL10 protein spanning amino acids from 115 to 145, including Q137P, Q137K, T143L, T143A, T144A, Q139H, A140W (Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice, Ku, Hsu, Carniol, Schmatz & Nielsen, 1999), A117E, P122R, and G124V (Aruna, Chakraborty, Rao, Santos, Ballesta & Sharma, 2005; Santos & Ballesta, 2002) were shown to be involved in sordarins resistance (Justice, Ku, Hsu, Carniol, Schmatz & Nielsen, 1999) (Table 6). The role of

this region in respect to sordarin activity was also verified by a study on the uL10 chimera protein. It showed that the region spanning amino acids 118-138 in the human uL10 protein, which corresponds to region 115-136 in yeast and especially residues at positions 119, 124, and 126, has an important role in determining resistance to sordarins (Santos, Rodriguez-Gabriel, Remacha & Ballesta, 2004) (Table 6). The important role of the uL10 protein is also underscored by the fact that the heterologous expression of the uL10 protein from *Dictyostelium discoideum* or *Rattus norvegicus* in a yeast strain lacking endogenous uL10 showed that the mammalian or protist protein conferred higher resistance to sordarin than the fungal one (Gomez-Lorenzo & Garcia-Bustos, 1998). Thus, the genetic analyses of the uL10 protein involved in resistance to sordarins indicated that uL10 provides valuable contribution to the sordarin mode of action (Gomez-Lorenzo & Garcia-Bustos, 1998). However, uL10 is in fact not involved in sordarin binding but in interaction with eEF2. Therefore, it was proposed that uL10 is rather involved in stabilization of the eEF2-sordarin complex on the ribosome as it belongs to the GAC (Briones & Ballesta, 2000). According to comparative functional studies of rRNA footprinting, the strongest rearrangement upon sordarin treatment was found in several rRNA positions: G1241, A1224, A1243, A1244, A1269, A1270, and A1272 and the α-sarcin loop in G3019 and G3025 indicating that the rRNA region in the GAC part is subjected to structural rearrangement and this region is responsible for eEF2 binding (Briones & Ballesta, 2000). Thus, it can be concluded that sordarin may act similarly to the thiostrepton antibiotic stalling the GAC region in the presence of eEF2 (Briones & Ballesta, 2000). On the other hand, analogous analysis with FA showed that FA protects rather than exposes equivalent nucleotides (Briones & Ballesta, 2000) indicating that, despite the homologous targets, these two antibiotics act in a different way with respect to translation factor EF-G/eEF2 (Briones & Ballesta, 2000).

Other P-stalk proteins such as P1 and P2 were also implicated in sordarin resistance. It was shown that in yeast which has four P1/P2 proteins (P1A, P1B, P2A, and P2B) deletion of the P1/P2 proteins may exert diverse effects on yeast cell sensitivity toward sordarin. Thus, deletion of either P1A or P2B reduced the resistance while deletion of either P1B or P2A did not have a significant effect. Deletion of both P1A and P2B had an additive effect whereas deletion of the other pair did not affect resistance (Table 6) (Gomez-Lorenzo & Garcia-Bustos, 1998). However, contrary to uL10 in which replacement of the yeast counterpart with its fungal *A. fumigatus* homolog directly influences strain sensitivity toward sordarin, the replacement of P1/P2 proteins in an analogous experiment did not change the yeast strain sensitivity indicating that the role of P1/P2 proteins is different than that of uL10 (Santos & Ballesta, 2002).

Besides, other ribosomal elements have an influence on sordarin activity (Figure 6). For example, deletion of the gene for ribosomal protein uL11 which is located close to uL10 increases the sensitivity of the yeast strain to sordarin; especially the lack of

the uL11B isoform is responsible for sensitivity to sordarin treatment (Wawiorka et al., 2016). uL11 is engaged in the elongation cycle by interplay with trGTPases (eEF1A or eEF2) and has an influence on the fidelity of translation and on eEF2-dependent translocation indicating that perturbations within the GAC not only increase resistance but may also cause sensitivity. According to the analysis of the translational half-transit time, the elongation cycle is significantly extended indicating that structural changes within the uL11 region can slow translocation and such a phenomenon may negatively affect eEF2 (Wawiorka et al., 2016). Another ribosomal element connected with the sordarin issue is the eL40 protein, also located in the GAC. Yeast mutants lacking eL40 displayed hypersensitivity toward sordarin (Fernandez-Pevida, Rodriguez-Galan, Diaz-Quintana, Kressler & de la Cruz, 2012).

3.3 eEF2 - diphthamide modification

Resistance of yeast cells to sordarin was also linked to a unique post-translational modification of eEF2, namely diphthamide modification (Botet, Rodriguez-Mateos, Ballesta, Revuelta & Remacha, 2008; Uthman et al., 2013). The diphthamidation pathway is a conserved pathway in eukaryotes and archaea, but not in eubacteria (Mayer et al., 2019), resulting in specific posttranslational modification of eEF2 at the H699 residue (Botet, Rodriguez-Mateos, Ballesta, Revuelta & Remacha, 2008). The diphthamide residue addition is dependent on the set of enzymes Dph1-Dph7 (Schaffrath & Stark, 2014). It has been shown that sordarin resistance of yeast strains is significantly increased when the diphthamidation pathway is defective by deletion one of the *dph* genes individually (Botet, Rodriguez-Mateos, Ballesta, Revuelta & Remacha, 2008; Villahermosa, Knapp & Fleck, 2017). This indicates that the diphthamide modification of eEF2, which is thought to be important for reading-frame maintenance on mRNA during translocation (Pellegrino et al., 2018), may probably allosterically cooperate with sordarin action and a lack of diphthamide abolishes sordarin sensitivity of fungal strains (Schaffrath, Abdel-Fattah, Klassen & Stark, 2014). Importantly, it was shown that, opposite to the sordarin-resistant mutants in relation to eEF2 which have a mutation within the amino acid region 518-524 (displaying almost no sordarin binding), the sordarin binding rate in *Δdph* mutants was as effective as for the wild-type yeast strain. Thus, it was proposed that the lack of diphthamide modification could affect the structure of eEF2 and the binding rate of the factor to the ribosomal particle, as it was also proposed for the uL10 protein with mutations within *N*-terminal domain (Botet, Rodriguez-Mateos, Ballesta, Revuelta & Remacha, 2008).

3.4 Additional elements modulating cell sensitivity toward sordarin

Besides the main elements, such as eEF2 representing the primary target for sordarin and ribosomal proteins uL10, uL11 and eL40, the genetic screen revealed a set

of genes related to sordarin sensitivity or resistance. 104 genes were associated with sordarin action involved in numerous biological process including: peptidyl-diphthamide biosynthesis protein biosynthesis with numerous ribosomal proteins genes, genes coding proteins involved in general catabolism genes encoding proteins connected with cell wall organization and biogenesis mitochondrial genome maintenance stress response, and RNA metabolism (Botet, Rodriguez-Mateos, Ballesta, Revuelta & Remacha, 2008). Although the identified genetic elements are not the main targets of sordarin, their lack may influence the sordarin sensitivity or resistance indirectly by modification of numerous metabolic pathways, e.g. triggering indirect factors such as inhibitor uptake through cell walls and membranes, drug consumption and delivery, and bypassing alternate pathways (McDermott, Walker & White, 2003).

Thus, it can be concluded that perturbations within the GAC element on the 60S ribosomal subunit, being at the same time the landing place for eEF2, mainly affect cell sensitivity toward sordarin (Figure 5).

4 Sordarin binding model and mechanism of inhibition

4.1 Sordarin binding mode with eEF2

As shown by biochemical analyses (Capa, Mendoza, Lavandera, Gomez de las Heras & Garcia-Bustos, 1998), the sordarin binding site is located on eEF2 (Capa, Mendoza, Lavandera, Gomez de las Heras & Garcia-Bustos, 1998; Justice et al., 1998). The genetic scanning mutagenesis which allowed removal of the functional side chain of particular amino acid residues without changes in the amino acid backbone structure showed that amino acid residues 517-524 were defined as the most critical ones and called a “sordarin-specificity region” - SSR. In particular, amino acids Y521 and S523 were recognized as the most essential (Shastry et al., 2001). However, with the advent of protein structural technologies like X-ray diffraction and single particle three-dimensional cryo-electron microscopy (cryo-EM) (Abeyrathne, Koh, Grant, Grigorieff & Korostelev, 2016), the structural model of sordarin bound to translational machinery elements was solved providing insight into the atomic resolution of the sordarin *modus operandi* (Andersen, Nissen & Nyborg, 2003). All structural models can be divided into two main groups; the first one comprises the structures of sordarin in a complex with eEF2 (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003; Jorgensen et al., 2004; Soe et al., 2007) and the second one includes the structure of 80S ribosomal particles together with eEF2 and sordarin (Abeyrathne, Koh, Grant, Grigorieff & Korostelev, 2016; Gomez-Lorenzo et al., 2000; Pellegrino et al., 2018; Spahn et al., 2004; Taylor, Nilsson, Merrill, Andersen, Nissen & Frank, 2007). The first structural insight into the sordarin-eEF2 complex was provided by X-ray crystallographic analyses showing the eEF2·sordarin structure at resolution of 2.9 Å (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003). The analysis provided several 3D

models of eEE2, including free apo-eEF2 and eEF2 in a complex with sordarin (eEF2·Sor). The apo-eEF2 consists of six structural domains: residues 2–218 and 329–345 (domain I or G-domain), 219–328 (G′-domain), 346–481 (domain II), 482–558 (domain III), 559–726 and 801–842 (domain IV), and 727–800 (domain V) (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003) (Figure 6 A). Overall, the apo-eEF2 complex has a packed structure; especially domains III, IV, and V form a compact arrangement while domains G/G' and II form a rigid separated element (Figure 6 A). On the other hand, the eEF2·Sor complex shows substantial structural rearrangements; nevertheless, the individual domains maintain their structural organization but change position in respect to each other (Figure 6 B-D). Thus, only minor conformational changes occur within the three G/G' and II *N*-terminal domains, maintaining the compact arrangement (Figure 6 A, B and C), while the three domains located at the *C*-termini do not form a rigid structure adopting a new extended arrangement, very distinct from that of apo-eEF2 (Figure 6 A-C). The most prominent changes are related to domains III, IV, and V which rotate in respect to the other domains; the rotation is as large as 75° leading to the so-called open conformation of the eEF2·Sor complex, compared to apo-eEF2 (Figure 6 C) (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003). In addition, upon sordarin binding to eEF2, domains III and V lose the inter-connecting interface with domains I and II and have less extensive interaction with domain IV (Figure 6 B). The binding structures of sordarin and its analogues (moriniafungin and sordarin derivative compound 1) to eEF2 are the same and resemble the one for eEF2·Sor with identical domain rearrangements (Figure 6 B) (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003; Soe et al., 2007). Therefore, the binding of sordarin is a remarkable example of an induced fit mechanism, inducing massive domain rearrangement in eEF2, especially domains III, IV, and V *versus* the other domains.

The sordarin binding pocket is located between domains III and V. All amino acid residues involved in sordarin binding are located in interdomain linkers, explaining the structural rearrangement induced by sordarin (Figure 6 E, F). The critical element that has been assigned by genetic/biochemical analyses to be involved in sordarin resistance, i.e. region 518-524 (sordarin specificity region - SSR), forms a β-strand within domain III and plays an important role in the formation of an interface element between domains III and V. The SSR forms an entrance to the sordarin binding pocket of eEF2 (Figure 6 E, F). The sordarin binding is coordinated by four amino acid side chains, Gln490, Glu524, Ala562, and Phe798 (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003) (Figure 6 F). The determined structures of sordarin derivatives, moriniafungin or sordarin compound, showed a similar binding pattern with the tetracyclic diterpene coordinated in a pocket formed by residues from domains III and V of eEF2, fixing the translation factor in the extended conformation (Soe et al., 2007)

(Figure 6. B). On the basis of structural and computational analyses it can be concluded that overall hydropathy indexes of numerous amino acid residues play an important role in sordarin binding and specificity at the same time. For example, in yeast, SSR has a hydrophobic pattern while the corresponding human SSR element displays a hydrophilic propensity showing that the hydrophobic elements forming SSR in yeast favor sordarin binding. Gln490 and Ala562 of yeast eEF2 are mutated in humans to equivalent Arg506 and Ser578 respectively, changing the hydrogen-bonding network which is unfavorable for sordarin binding. Thus, it has been shown that, in human eEF2, the different amino acid side chain composition within SSR and in other amino acid substitutions at the biding pocket change the drug-binding cavity drastically making it different from its fungal counterparts; hence human eEF2 is unable to bind sordarin (Chakraborty, Mukherjee & Sengupta, 2013).

4.2 80S-eEF2 complex

eEF2 represents the primary target for sordarin; however, since sordarin is centered on eEF2, it induces broad allosteric structural rearrangement affecting the performance of the translational machinery exclusively. The eEF2·sor complex with the ribosome represents a functional entity which has been visualized by numerous structural approaches, especially with the aid of cryo-electron microscopy, providing functional insight into the sordarin *modus operandi*. The first 3D structural model emerged was the 80S ribosome·eEF2 complex with sordarin GM193633 solved at 17.5 Å resolution (Gomez-Lorenzo et al., 2000) and the structure was further improved at 11.7 Å resolution (Spahn et al., 2004). The structure of eEF2·sor with the 80S·eEF2 complex was in line with earlier reports (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003; Pellegrino et al., 2018) indicating that eEF2 within the 80S complex possesses a unique conformation arrangement upon ribosome binding (Figure 7. A), i.e. an extended conformation. The structural insight showed transition from free apo-eEF2 to eEF2·80S involving rotation of domains III, IV, and V relative to domains I and II, closely resembling the free eEF2·sordarin structure determined by the X-ray approach, yet having an intermediate state (Figure 7. B). The interplay between eEF2 and the 80S ribosome involves interaction with both ribosomal subunits and all five domains of the factor are engaged. eEF2 forms extensive interactions with the GTPase-associated center (GAC). Domain I interacts with the 25S rRNA – the sarcin-ricin loop (SRL) and additionally with ribosomal proteins uL6 and the base of the stalk including the uL10 and uL11. Domain II contacts with 18S rRNA, domain III binds to SRL and uS12, domain IV binds to 25S rRNA, approaching the decoding center - DC, and domain V forms interactions with 25S rRNA and uL11 (Figure 7 A) (Spahn et al., 2004). The conformational alteration observed within eEF2·sordarine·80S indicates that sordarin binding stabilizes rearrangement within eEF2 domain III, fixing it in the intermediate

state (Figure 7. C). This induced sordarin-binding affinity of eEF2 for the ribosome is increased because domain III on the ribosome adopts a conformation different from free apo-eEF2, free eEF2·sordarin, and eEF2·ribosome (Figure 7. C) indicating that sordarin induces a non-canonical domain III state within the eEF2·sordarine·80S complex (Spahn et al., 2004). The binding mode of eEF2 within the 80S ribosome in the complex with sordarin was elucidated at the atomic level by determining the structure of the complex formed using *S. cerevisiae* 80S ribosomes with Taura syndrome virus IRES RNA and eEF2 in the complex with GTP and sordarin. The analysis provided five distinct 80S·IRES·eEF2·GDP·sordarin structures at resolutions of 3.5 to 4.2 Å, sufficient to resolve individual residues in the core regions of the ribosome and eEF2 (Abeyrathne, Koh, Grant, Grigorieff & Korostelev, 2016). In all presented structures, eEF2 is rigidly attached to the GAC of the 60S subunit (Figure 8. A). The most striking observation regarding the eEF2 interaction with 80S is the involvement of the P-stalk base formed by uL10. An αββ motif of the uL10 protein (amino acid residues 126–154 – mutations within this element confer resistance to sordarin) is packed into the α-helix D (amino acid residues 172–188) of the G domain and the β-sheet region (amino acid residues 246–263) of the G' insert of eEF2, stabilizing the G/G' domain (Figure 8, A, inset). Importantly, the base of the uL10 P-stalk remains unchanged in all structures indicating that the G/G' domain adopts a fixed invariant state. However, with respect to the stalk base position in the 80S complex in the absence of eEF2 (Koh, Brilot, Grigorieff & Korostelev, 2014; Svidritskiy, Ling, Ermolenko & Korostelev, 2013), the uL10 P-stalk base is shifted by ~13 Å toward the A site indicating that the uL10 base undergoes structural rearrangement upon the eEF2 binding, locking eEF2 within the 80S. Thus, the stalk base together with SRL forms clamps which position the G/G' domain within the GAC (Figure 8, A and B, upper panel). This stabilization forces the GAC to adopt a GTP-bound conformation, resembling the states observed for additional trGTPases in the presence of GTP analogs (Voorhees, Schmeing, Kelley & Ramakrishnan, 2010). On the other hand, the fully rotated 40S subunit of the pre-translocation ribosome provides an interaction surface for the other domains complementing the P-stalk and SRL for eEF2 binding. As already shown by either X-ray crystallography or cryo-EM, the most pronounced inter-domain rearrangement in eEF2 involves movement of domain III in respect to domain V. Structural analysis showed that in the rotated state of 40S during the translocation step domain III is associated with domain V while the G/G' domain does not undergo noticeable rearrangements. Upon structural transitions during translocation the most pronounced structural changes are related to helix A of domain III which is displaced toward domain I (Figure 8. B, inset). This displacement is caused by the movement of the 40S body; especially the ribosomal protein uS12 contributes to this change during the last step of translocation. Thus, the most particular structural transition during translocation is the

shift of domain III by uS12 which initiates intra-domain rearrangements in eEF2 by unstacking domain III from that of domain V. Such rearrangement may induce a conformational transition leading to characteristic structure of free apo-eEF2, adopting a compact structure with low affinity for the unrotated 80S. The observed structural transitions laid the first foundation for elucidation of the sordarin *modus operandi*, showing perturbations caused by sordarin in the structural transition trajectory from pre-translocation to post-translocation structures of eEF2·sordarine·80S complexes. Thus, it was proposed that eEF2 in the sordarin bound state has domain III shifted in a way that it stabilizes the interface between domains III and V, keeping it unchanged during translocation. Thus, sordarin stabilizes the interactions between domain III and V, and the presence of sordarin may interfere with the final stages of reverse rotation of the post-translocation ribosome, preventing the reverse rotation of 40S and the release of GDP-bound eEF2 at the same time. Sordarin stabilizes the interdomain interactions between domains III and V and blocks the uS12-induced disengagement of domain III from domain V (Figure 8. B, inset); however, sordarin does not block GTP hydrolysis (Abeyrathne, Koh, Grant, Grigorieff & Korostelev, 2016).

The sordarin action was further elucidated by determination of a set of 80S structural models in a complex with mRNA, cognate tRNA, eEF2, and GMPPCP, i.e. a non-hydrolyzable analog of GTP. Especially two complexes are of great interest: the 80S complex with GDP and aluminum fluoride (AlF_4^-) instead of GMPPCP, as GDP-AlF_4^- traps 80S ribosome-bound eEF-2 in a transition-like state just after GTP hydrolysis. Importantly, the complex was supplemented with sordarin. The second one is the 80S ribosome complex with a GMPPCP/sordarin complex designed to provide understanding of the drug binding when eEF2 is bound to the ribosome in a GTP-like state (Pellegrino et al., 2018). All resolved structures corresponded to the states of translocating ribosome, showing the intermediate of “unlocked” fully rotated 40S with extended anti-clockwise head swiveling induced by eEF2. Overall, the eEF2 domain arrangement resembled that observed in other structural models displaying an extended structure, especially fixed by extensive interactions within the GAC (Figure 9). Especially, the ensemble of available structures provides insight into the action of domain IV of eEF2 which carries a unique post-translational modification, namely with diphthamide covalently bound to a conserved histidine residue (His699 in yeast) which forms the very tip of domain IV. Additionally, the mutation within this residue has been shown to confer resistance to sordarin. The arrangement of domain IV before GTP hydrolysis, especially H699 with diphthamide modification, shows that the diphthamide of eEF2 is pointing toward the mRNA path, so called “outward” orientation (Figure 9. A and B) suggesting that when eEF2 is bound to the 80S ribosome in the GTP-like state diphthamide can act as a “pawl” providing tight interaction with mRNA, preventing slippage or frameshifting of mRNA during translocation, hence

ensuring the fidelity of translocation as proposed earlier by biochemical analyses (Liu et al., 2012). On the other hand, striking data are provided by the 80S·GMPPCP·eEF2·sordarin structure which show that, upon sordarin binding to the eEF2 in GTP state, structural rearrangements within domains III and V exert distal effect on the very tip of domain IV. Namely, His699 with diphthamide changes orientation and points away from the mRNA within the DC, the so-called “inward” orientation (Figure 9. C and D). This indicates that, by indirect action on diphthamide, sordarin may stabilize the GTP bound-state of eEF2 additionally contributing to the lock of the factor on the ribosome. Additionally, based on the 80S structure with GDP/AlF_4^-, immediately after GTP hydrolysis but before phosphate release, the tip of eEF2 domain IV with the diphthamide residue is rearranged into an intermediate conformation and points toward rRNA helix 44 on the 40S which forms the core of DC, substantially distorting the interaction network within the DC arrangement (Figure 9. E and F) (Pellegrino et al., 2018). Thus, considering the post-GTP-hydrolysis state of eEF2 in respect to domain IV, sordarin induces and stabilizes the unusual structural intermediate state at the tip of domain IV influencing DC which may additionally contribute to stalling of eEF2 on the ribosome. Therefore, it can be concluded that sordarin acts in an allosteric way and structural rearrangements within domains III and V induced by sordarin are also conveyed to the tip of domain IV where His699 with diphthamide is located, distorting DC and contributing to stalling of eEF2 on 80S. Importantly, the structural analyses are in line with biochemical data showing that stabilization of eEF2 can take place irrespective of the GTP/GDP state (Dominguez, Gomez-Lorenzo & Martin, 1999).

5 Mechanism of sordarin inhibition

Translation represents a highly conserved metabolic cycle in all cells consisting of several steps including initiation, elongation, termination, and recycling with central element the ribosome as a nano-machine which harnesses Brownian motion, coupling spontaneous conformational changes driven by thermal energy to directed movement facilitated by trGTPases (Frank & Gonzalez, 2010). The elongation cycle lies in the heart of the translational cycle, consisting of decoding, peptide bond formation, and translocation steps (Figure 10). The elongation cycle starts with the binding of eEF1A·GTP·aminoacyl-tRNA as the so-called ternary complex to the A site of the translationally competent 80S ribosome with the P site occupied by peptidyl-tRNA (Figure 10. I). The decoding step is driven by anticodon-codon duplex formation between aminoacyl-tRNA and mRNA and structurally verified by the rRNA of the decoding center. The accommodation of the ternary complex with cognate aminoacyl-tRNA induces ribosome-dependent GTP hydrolysis catalyzed by eEF1A which constitutes the turning point, allowing the aminoacyl-tRNA to be fully accommodated

into the A site while eEF1A·GDP is released from the ribosome (Figure 10, II). The aminoacyl-tRNA accommodation is immediately followed by peptide bond formation where the amino acid moiety of aminoacyl-RNA reacts with peptidyl-tRNA and the nascent polypeptide chain is extended by one amino acid residue (Figure 10. III). Consequently, the nascent peptide chain is transferred to A-site tRNA, leaving deacylated tRNA in the P site (Figure 10. III). At this stage, the ribosome changes the structural rearrangement and all tRNAs adopt the so-called hybrid state with peptidyl-tRNA in A/P and free tRNA in P/E position. The hybrid state induces rotation of the small ribosomal subunits by 6^{o} with respect to the large subunit, called a 'rotated or ratcheted' ribosome (Figure 10. IV). Before the next round of peptide elongation, tRNAs and mRNA should be moved along the ribosome in the process called translocation where mRNA shifts by one codon, exposing a new nucleotide triplet in the A site (Dever, Dinman & Green, 2018). During hybrid state (which is prerequisite for translocation), the ribosome oscillates spontaneously between two states: the pre-translocational state (rotated) and the post-translocational state (unrotated) which represent an intrinsic structural propensity of the ribosome driven by Brownian motions and based on thermal energy (Frank & Gonzalez, 2010). The translocation is facilitated by trGTPase-eEF2 which recognizes and binds to 80S and stabilizes the rotated conformational state of the ribosome (Figure 10. V). At the same time, it promotes a conformational rearrangement of the 40S subunit by inducing the head swivel which leads to 'unlocking' of the 40S head-body interactions with 60S and accelerating the rate-limiting step of translocation: the movement of tRNAs and mRNA on the small ribosomal subunit at the cost of GTP hydrolysis catalyzed by eEF2 (Figure 10. VI). eEF2 can be regarded as a 'doorstop' allowing movement of the tRNAs·mRNA module throughout A, P, and E sites which leads to exposition of a new codon in the A site to the ribosome with concomitant release of eEF2·GDP from the ribosomal complex (Figure 10. VII) (Dever, Dinman & Green, 2018).

The sordarin *modus operandi*, specifically centered on eEF2, blocks the very last step of the elongation cycle, namely the translocation step and thus does not allow resetting the translational machinery system for the next round of elongation. The following sequence of events for the eEF2 action regarding the sordarin inhibition effect can be proposed: eEF2 is a five-domain protein with two so-called super-domains. The first domain I/II (also regarded as G and G' domains) is responsible for GTP hydrolysis and has been shown to interact firmly with the ribosomal GAC anchoring EF2 to 80S. The second super domain, consisting of domains III-IV-V, represent a structural entity undergoing the most significant structural changes directly participating in translocation, interacting with the ribosomal A site, reaching at the same time the decoding center (Spahn et al., 2004). After decoding and peptide bond formation, the ribosome is in the hybrid state and at the same time in the pre-translocation state and

can be regarded as a substrate for the eEF2·GTP complex (Figure 10. IV). Sordarin may bind to the eEF2·GTP complex already in the cytoplasm and the complex in the presence of sordarin is adopting extended conformation which can bind the rotated 80S. Upon binding to the ribosome eEF2·GTP·sordarin is accommodated in such a way that super-domain I/II is trapped by the GAC elements (ribosomal proteins uL11, uL6, and uL10 and rRNA – SRL, Figure 8 A). Super-domain III-IV-V is inserted into the A site, with domains III and V of eEF2 anchoring the factor to the ribosome through interactions with uS12 and uL11/uL10 in the 40S and 60S subunits, respectively (Figure 8). Domain IV points directly toward the decoding center with the invariant His699 with diphthamide modification acting as a "pawl" and preventing slippage of mRNA and frameshifting, however in the presence of sordarin, the decoding center is distorted and such structural aberration provides stalling force for eEF2. Accommodation of eEF2 and stabilization of the rotated state of the ribosome lead to induction of GTP hydrolysis within the I/II super domain which is usually (without sordarin) communicated to domain III and cause structural rearrangement in the interface domains between the I/II and III/V and within domains III/IV shown as an extended conformation which further leads to the release of eEF2·GDP. It is assumed that GTP hydrolysis contributes to the movement of domain IV which allows it to adopt the favored conformation of the post-translocational state. However, in the presence of sordarin such arrangement is induced by the antibiotic, without affecting GTP hydrolysis (Figure 9). Finally, the transition of the ribosome to the unrotated state initiates the uS12-induced disengagement of domain III from domain V and the super-domain III-IV-V loses its structural integrity adopting compact apo-eEF2·GDP which allows it to leave the ribosome (Figure 10. VII)). However, in the presence of sordarin, which has the binding site at the interface of domains III and V, it induces and provides stabilization forces for the extended conformation of eEF2 (Figure 10. alternative pathway). Thus, upon binding to the fully rotated, eEF2 in a complex with sordarin adopts a functional extended conformation which allows GTP hydrolysis and translocation. However, sordarin maintains the stiffness of eEF2 by preventing disengagement of domain III from V and by changing the position of the tip of domain IV where diphthamide disturbs the decoding center, contributing to the stalling of eEF2 on the ribosome (Figure 10).

6 Prospect

Sordarin represents a unique and promising inhibitor of fungal growth and may help to combat human infections with extraordinary specificity and exceptional low toxicity. With its unique mechanism of action among anti-fungal compounds, e.g. binding to fungal eEF2 exclusively, sordarin targets the primary metabolic cycle such as translation, making this compound a superior antibiotic compared to other antifungal

compounds (Carrillo-Munoz, Giusiano, Ezkurra & Quindos, 2006). Therefore, the sordarin application should be extended from a useful tool in eukaryotic translation system research to clinical therapies of fungal infections. To achieve the application of sordarin as a useful antibiotic, there are some points to be considered. Firstly, the chemical properties should be improved for better stability as sordarin is quickly decomposed/metabolized *in vivo*. Secondly, the selectivity may also represent an issue as there is no compound with broad specificity toward all pathogenic fungal species. Thirdly, based on *in vitro* and *in vivo* studies, sordarin metabolism and energy network interaction should be explored to provide knowledge of its fate in the cell and cast light on its stability. Fourthly, an industrial production method with low expense and high efficiency has to be developed as it is currently produced on a low scale. To sum up, sordarin represents a class of antifungal antibiotics with exceptionally high application potential but its clinical application is far from being well developed, especially in terms of its stability and broad specificity. Therefore, there is a need to carry out comprehensive research on sordarin as there is a gap on the way from the laboratory to medical applications which requires further refinement.

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Figures

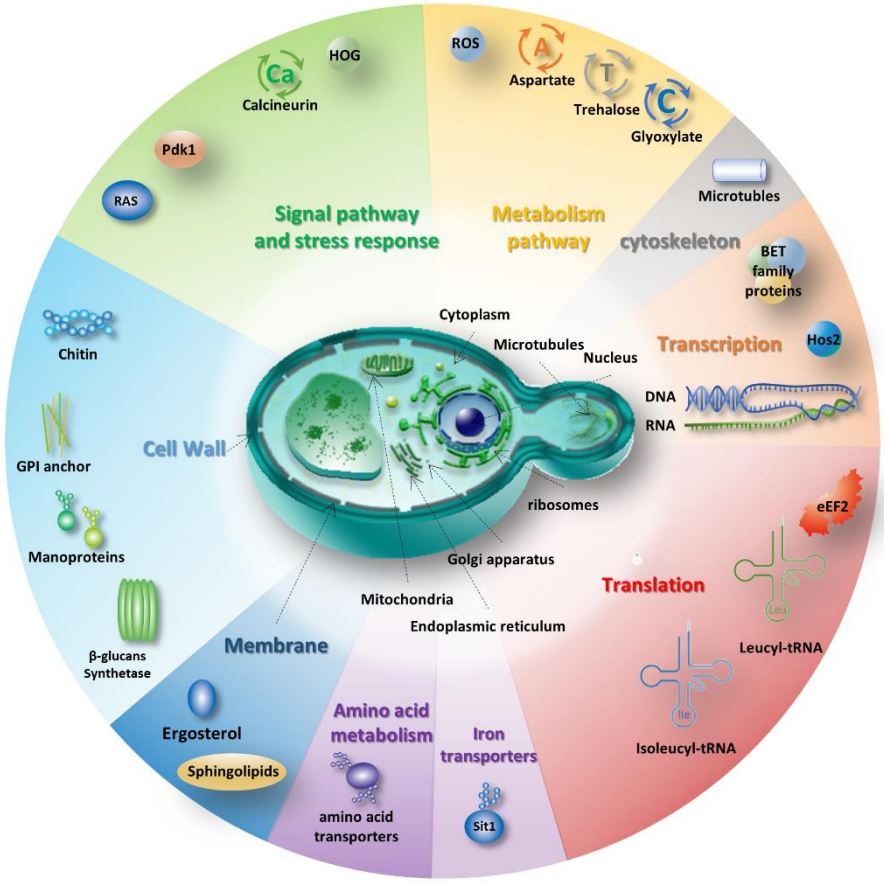

Figure 1 Cellular targets of antifungals

The major metabolic pathways with particular cellular components being targeted by antifungal chemicals are shown. The following pathways/cellular components are presented: the cell wall with specific elements, β-glucan synthetase, mano-proteins, GPI anchor and chitin metabolism; membrane metabolism with ergosterol metabolism and sphingolipids synthesis; amino acid metabolism with amino acid transporters as a target; siderophore iron transporter with the Sit1 protein; translation with isoleucyl-tRNA, leucyl-tRNA synthetases, and elongation factor 2 (eEF2) as targets; transcription with DNA and RNA synthesis pathways, histone deacetylase 2 (Hos2), and chromatin-interacting modules with bromodomain and extra-terminal (BET) family proteins are also targeted by antifungals; cytoskeleton with microtubules biosynthesis pathway; general metabolism pathways are targeted by a vast number of antifungals including the glyoxylate cycle, trehalose pathway, and aspartate synthesis pathway, reactive oxygen species (ROS), and oxidative damage; signal transduction pathway and stress response system are also considered as targets for antifungals, with such targets as the RAS pathway, 3-phosphoinositide-dependent protein kinase 1 (Pdk1) pathway, high osmolarity glycerol (HOG) pathway, and calcineurin pathway.

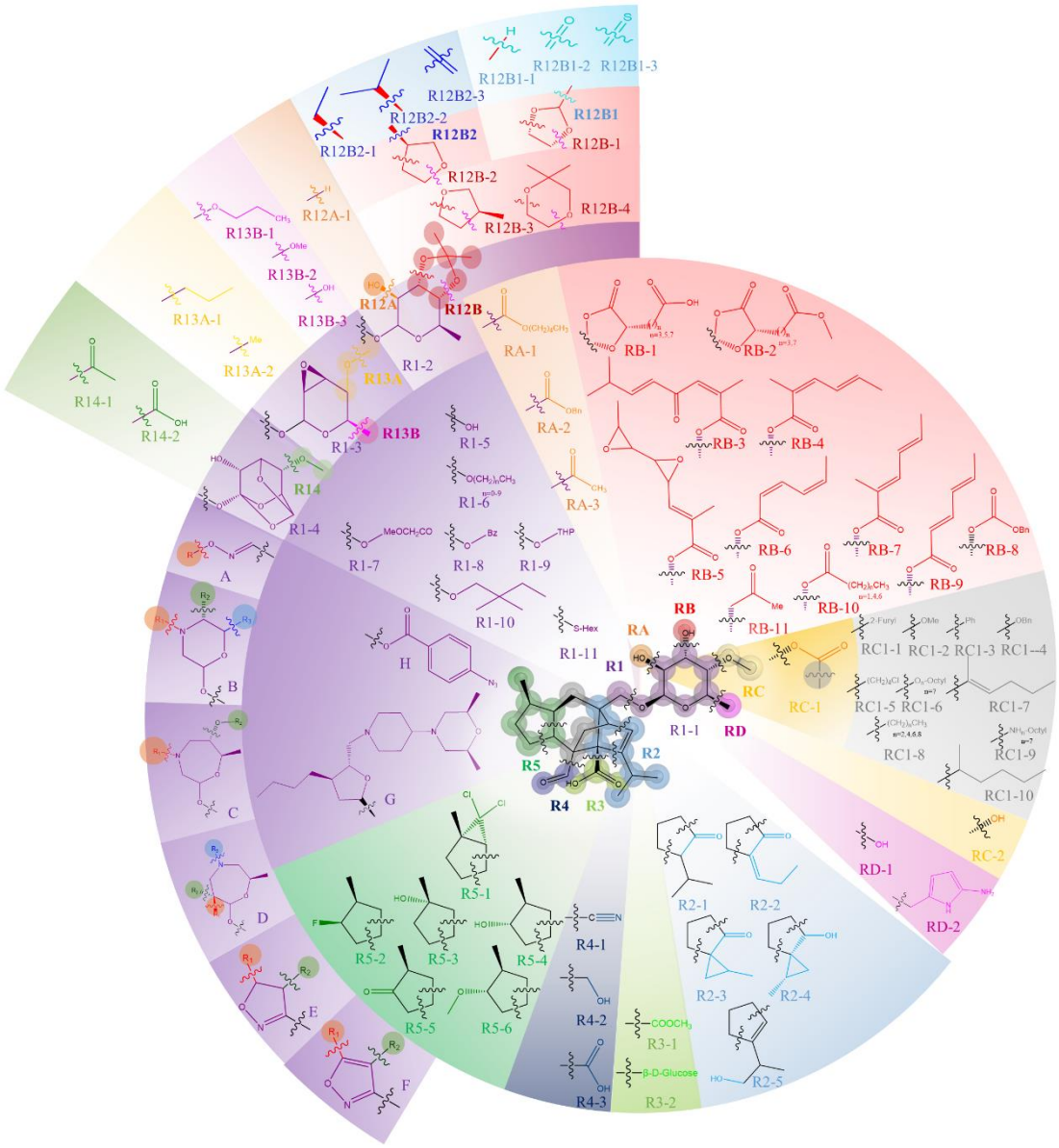

Figure 2 Sordarin structure and derivatives

The sordarin structure is presented as an integrated model with a core element in the center, and subsequent residues are labeled in colors. The labeled elements are as follows: R1 (purple) - glycosides; R2 (blue) - five-membered ring containing an isopropyl group; R3 (light green) – carboxyl group; R4 (dark blue) - formyl group; R5 (dark green) - five-membered ring with a methyl group; elements without additional modification are labeled in gray. Within the R1 group, the R1-1 element can be recognized with four residues that can be modified: RA (orange), RB (red), RC (yellow), RD (pink). Sordarin derivatives with specific substitution within these groups are labeled from RA-1 to RA-3 and the same nomenclature applies to RB, RC, and RD; the wavy line shows the place of substitution. The whole R1 group can be substituted, described as R1-2 to R1-11. The additional derivatives extending the variability of known modifications are shown as additional layers. R1-2 can have additional substitutions designated as R1-2A, R1-2B, and R1-2B, with further extensions; the

additional derivatives of R1-3 and R1-4 are marked as well. The derivatives of the R2 moiety are shown as R2-1, R2-2, R2-3, R2-4, and R2-5. The R3 moiety has two substitutions R3-1 and R3-2. The R4 element extends to R4-1, R4-2, and R4-3. R5 has six modifications: R5-1, R5-2, R5-3, R5-4, R5-5, and R5-6. The additional sordarin group - azasordarin derivatives are shown as A-G structures, which replace the R1 moiety, and are further extended in figure 3. Natural sordarin structures: R1-1 sordarin B; sordarin C, R2-5; sordarin D, R2-1; sordarin E, R2-3; sordarin F, R2-2; zofimarin, RB-6; isozofimarin, RB-9; xylarin a (SCH57404), R1-4; xylarin b, R1-4; xylarin c, R1-4; GR 135402, RB-7; BE31405, R14-1; trichosordarin A , R2-4; moriniafungin B, RB-1 n=5; moriniafungin C, RB-1 n=3; moriniafungin D, RB-2 n=3; moriniafungin E, RB-2 n=7; moriniafungin F, RB-1 n=7, R4-3; moriniafungin G, RB-2 n=7, R4-3; sordaricin, R1-5; hypoxysordarin (FR231956), RB-5; hydroxysordarin, RD-1.

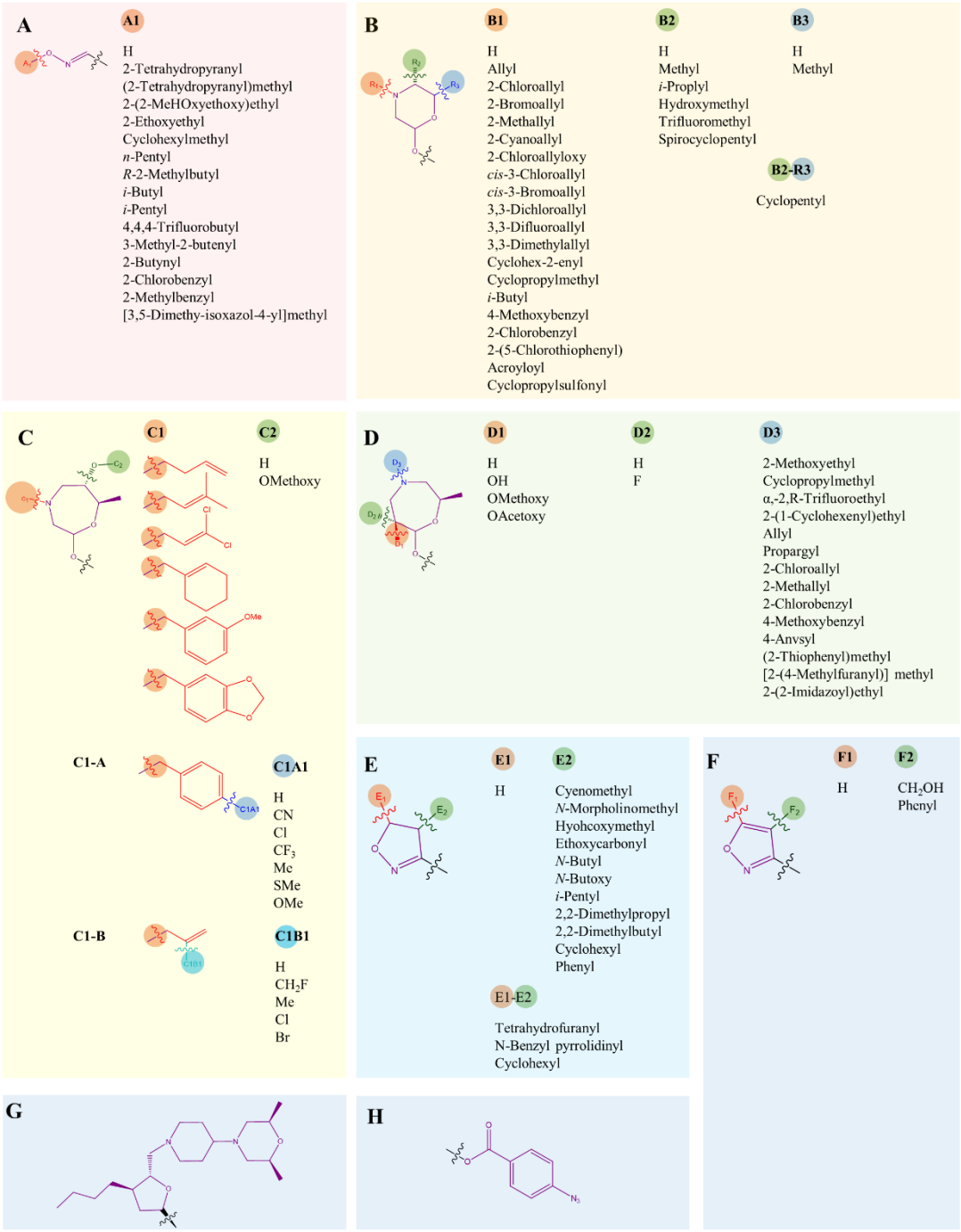

Figure 3 Azasordarin derivatives

Azasordarin derivatives shown as additional moieties are the replacement of the R1 residue shown in Figure 2. The black wavy line shows the place of substitution within R1. The cycles in particular colors represent additional substitutions within azasordarins; the color wavy line shows the place of substitution within particular azasordarin moieties. A - Sordarin oxime derivatives, A stands for residues (in orange) that are additionally present in oxime derivatives (Serrano-Wu et al., 2002b). B - Sordarin morpholino derivatives; the groups is extended to B1 (orange), B2 (green), and B3 (blue) (Serrano-Wu et al., 2003). C - N-substituted 1,4-oxazepanyl sordarins;

the group is divided into C1 (orange) and C2 (green) derivatives (Kaneko, Arai, Uchida, Harasaki, Fukuoka & Konosu, 2002). D - Oxazepine sordarins; three types of derivatives is recognized as D1 (orange), D2 (green), and D3 (blue) (Serrano-Wu et al., 2002a). E - Isoxazoline sordarins, R1(Red) and R2 (green) derivatives, additional derivatives are formed by linkage of R1 and R2 (Serrano-Wu et al., 2002b). F - Isoxazole sordarins with two additional moieties R1 (Red) and R2 (green) (Serrano-Wu et al., 2002b). G - Sordarin FR29581 containing a single substitution (Hanadate et al., 2009)

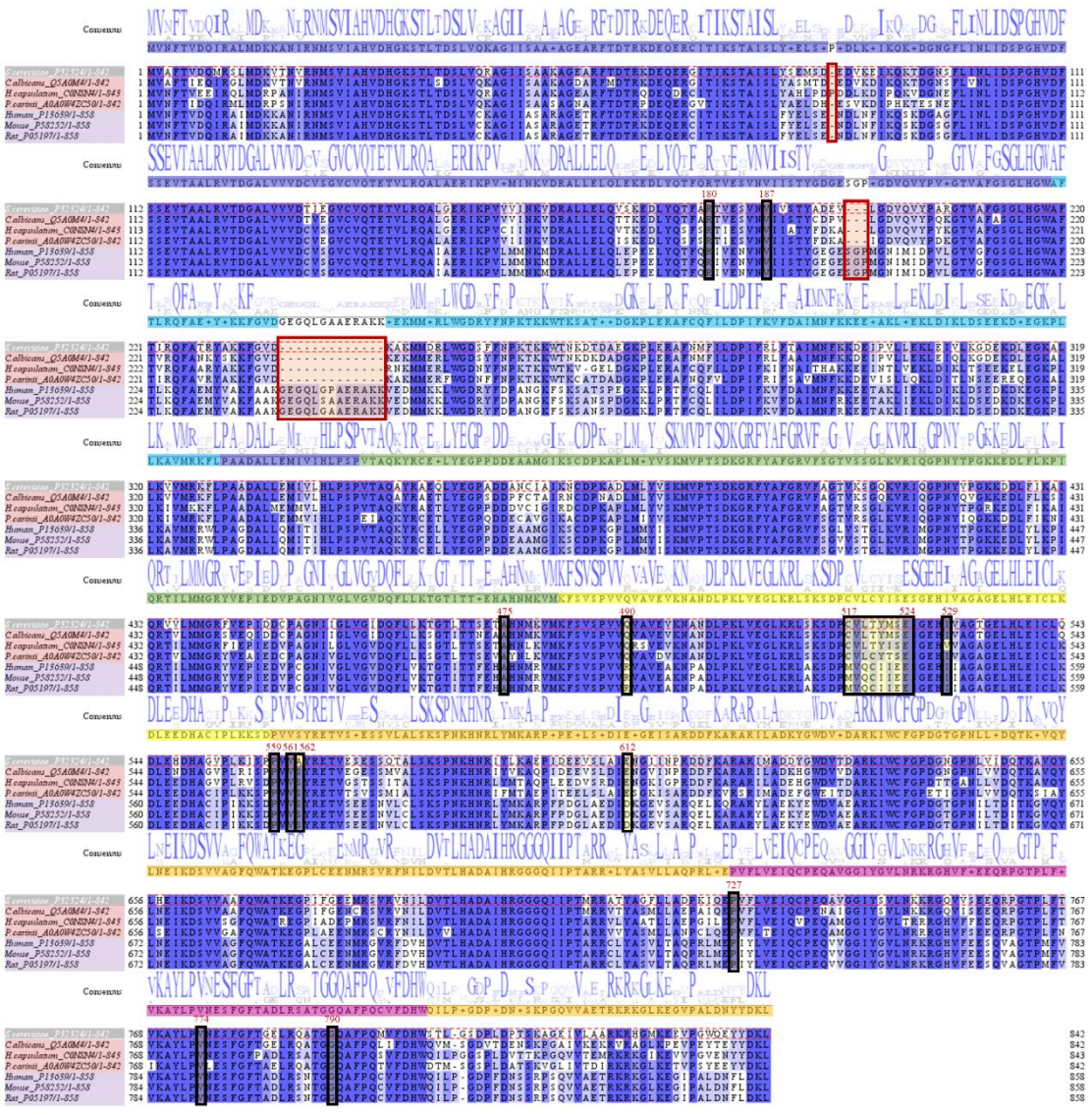

Figure 4 eEF2 amino acid alignment

The amino acid alignment represents a set of representative eEF2 sequences from several species: fungi - *S. cerevisiae* (uniport ID: P32324), *C. albicans* (uniport ID: Q5A0M4), *H. capsulatum* (uniport ID: C0NSN4), *P. cannii* (uniport ID: A0A0W4ZC50); mammals: human (uniport ID: P13639), mouse (uniport ID: P58252), rat (uniport ID: P05197). The alignment was conducted using Jalview (Version 2.11.1.3) [115]. The highly conserved and semi-conserved amino acid residues are labeled with dark blue and light blue, respectively. The amino acid residues involved in sordarin resistance are labeled with a black box. The insertion elements characteristic for mammalian eEF2 are labeled with a red box. The sequences are numbered according to the yeast *S. cerevisiae* sequence. The position of the eEF2 domains was labeled according to the *S. cerevisiae* structural model and is presented above the alignment

with the color code: blue for domain I, cyan for domain G', green for domain II, yellow for domain III, orange for domain IV, magenta for domain V. The consensus sequence is presented in a letter mode with the size of the letter depicting the strength of homology.

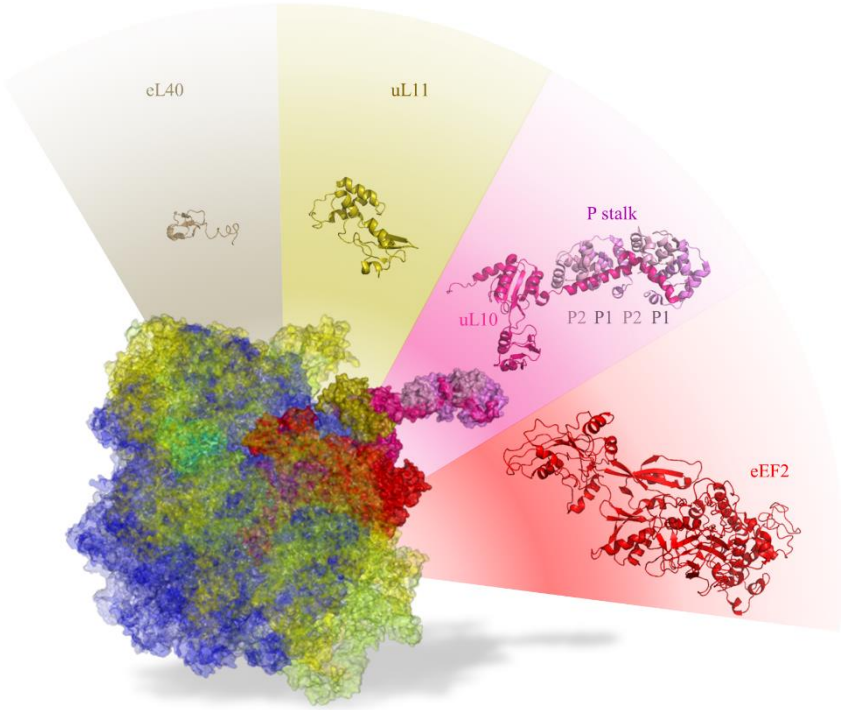

Figure 5 80S ribosome structure with proteins related to sordarin action

The central part shows the structure of the *S. cerevisiae* ribosome (complex 80S·eEF2·GMPPCP and with mRNA and tRNA, determined by cryo-EM (PDB:6GQV) (Pellegrino et al., 2018) presented as a so-called crown view in respect to the large ribosomal subunit. The 40S subunit is presented in a yellow semi-transparent mode, the 60S subunit - in blue. The individual ribosomal proteins involved in sordarin are marked in separate colors. eEF2 is marked in red. The stalk protein structures: uL11, uL10, and P-proteins are taken from the 80S structure (PDB:4V6I) (Armache et al., 2010) and implemented into the 6GQV structure to provide complete structural representation of the stalk; uL10 is shown in hot pink, P1 in violet, P2 in pink, uL11 in olive, uL6 in wheat, eL40 in sand, and uS12 in purple. All models were prepared with the PyMOL molecular graphics system software (Version 0.9 Schrödinger, LLC.) (Schrodinger, 2015).

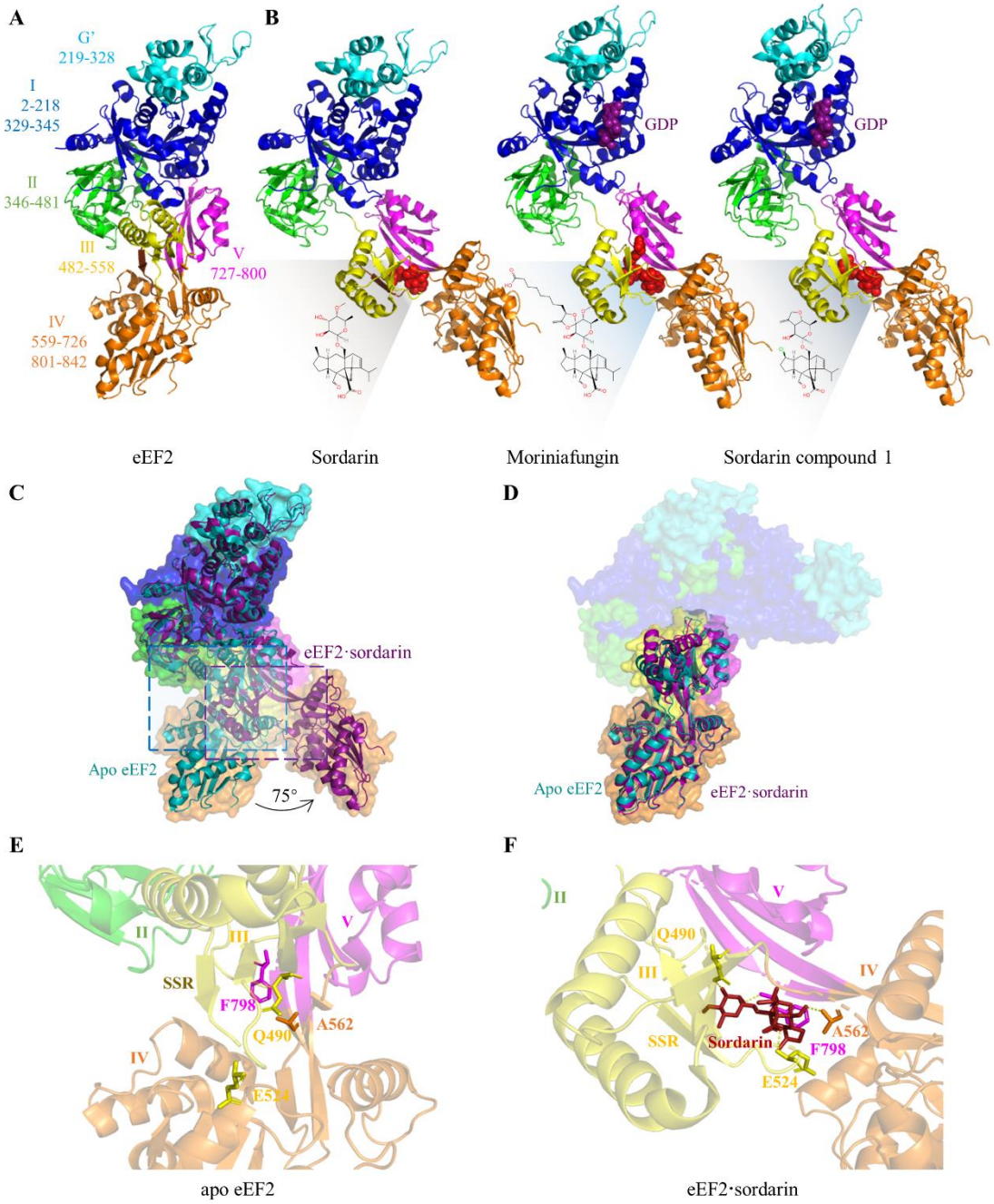

Figure 6 eEF2 structures with sordarin and its analogues

A - Structure of apo-eEF2 without sordarin (PDB:1N0V) (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003). The eEF2 individual domains are marked as follows: blue - domain I (G), residues 2-218 and 329-345; cyan - domain G', 219-328; green - domain II, 346-481; yellow - domain III, 482-558; orange - domain IV 727-800; magenta - domain V, 559-726, 801-842. B - Structure of eEF2 bound with sordarin (PDB:1N0U) (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen,

2003), moriniafungin (PDB:2NPF) (Soe et al., 2007), and sordarin derivative compound 1 (PDB:2E1R) (Soe et al., 2007). C - Structural alignment of apo-eEF2 and eEF2·sordarin, the domain I/II and G' are aligned as an invariant element. The arrow indicates the rotation of domains III, IV, and V by 75°; apo-eEF2 is marked in teal and eEF2·sordarin in purple. D - alignment of apo-eEF2 and eEF2·sordarin, domains III, IV, and V are aligned as an invariant element. E-F - eEF2 sordarin binding sites in apo-eEF2 and eEF2·sordarin enlarged from the region marked with boxes in C. The amino acid residues Q490, E524 in domain III, A562 in domain IV, and F798 in domain V near sordarin (red) and SSR are marked. All models were prepared with the PyMOL molecular graphics system software (Version 0.9 Schrödinger, LLC.)(Schrodinger, 2015).

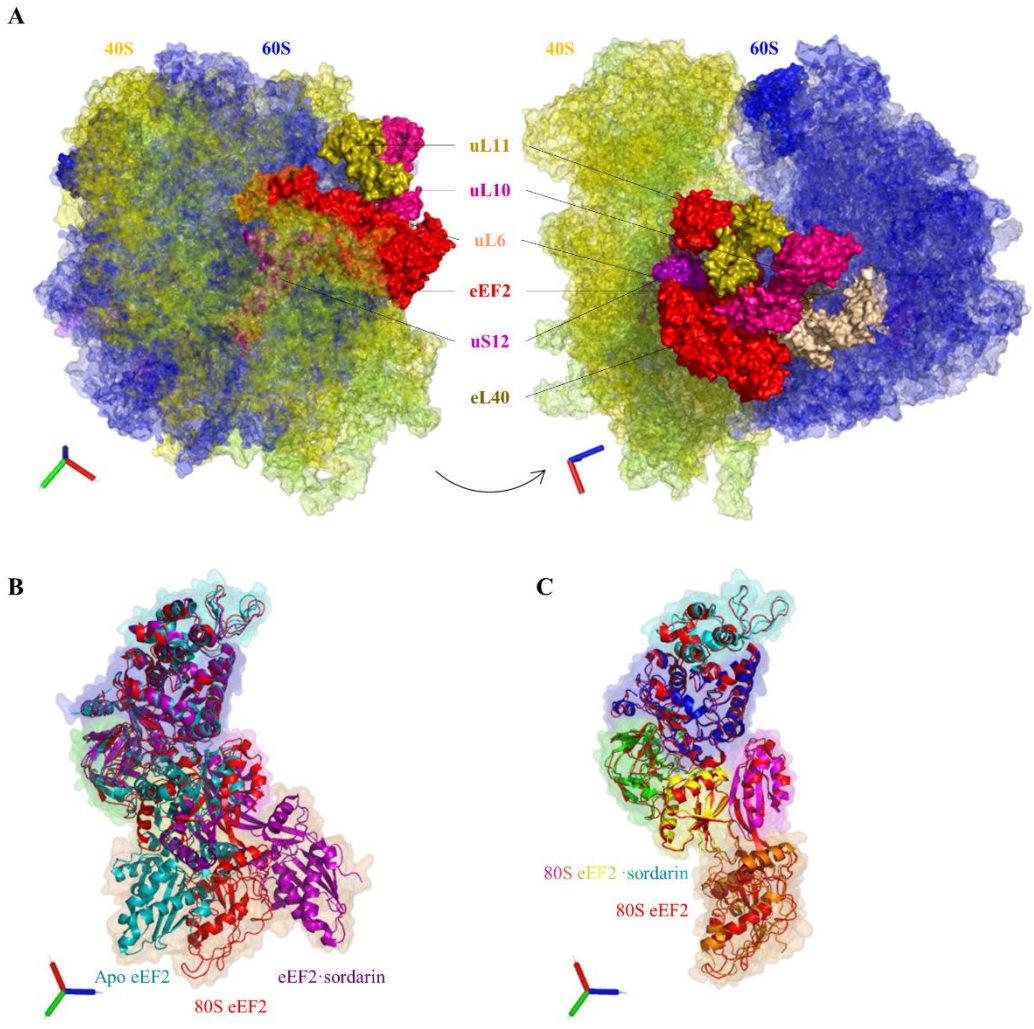

Figure 7 Yeast 80S ribosome in a complex with eEF2

A - the structure of the 80S·GMPPCP·mRNA·tRNA (PDB:6GQV) (Pellegrino et al., 2018) complex is shown as a crown view - left panel. The small ribosomal subunit is marked in yellow (in the front) and the large ribosomal subunit is marked in blue (in the back). The ribosomal proteins, which constitute the GTPase associated center

(GAC), are marked and labeled with colors accordingly. The ribosomal proteins constituting the GAC and involved in EF2 binding are as follows: uL6 in wheat, uL10 in hot pink, uL11 in olive, uS12 in purple, eL40 in sand, and eEF2 in red. The uL11 was separately implemented from the 80S structure (PDB:4V6I) (Armache et al., 2010) in order to present the whole GAC element composed of uL11 and uL10. The right panel - the 80S structure in a rotated view 315º around the Z axis and 270º around the Y axis. B - alignment of the three structures of eEF2: apo-eEF2 - teal (PDB:1N0V), eEF2·sordarin - purple (PDB:1N0U) (Jorgensen, Ortiz, Carr-Schmid, Nissen, Kinzy & Andersen, 2003), and eEF2 from 80S - red (PDB: 6GQ1) with I/II and G' domains in an invariant position. C - alignment of the two eEF2 structures; eEF2 in a complex with 80S without sordarin (PDB:6GQV) supplemented with GMPPCP - red and eEF2 in a complex with 80S with sordarin and GMPPCP (PDB:6GQ1) (Pellegrino et al., 2018). All domains are shown in multicolors as in figure 6.

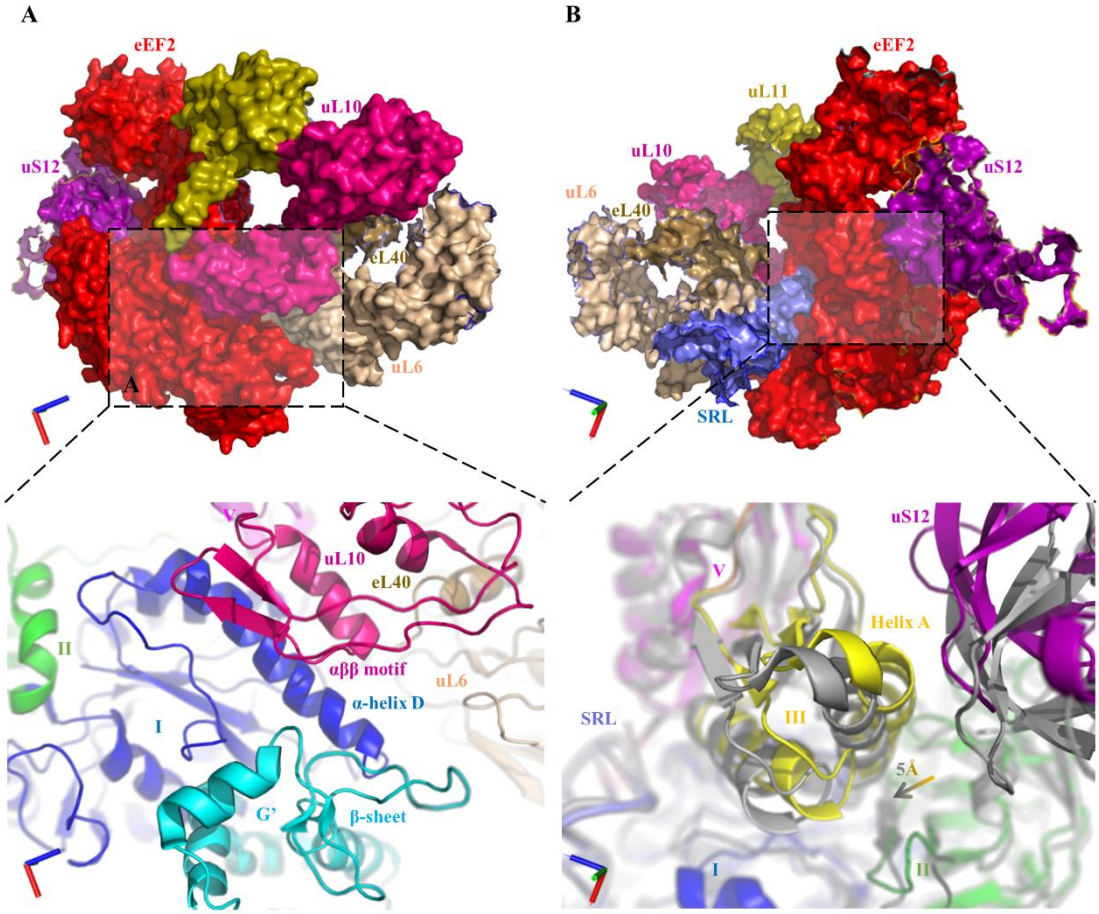

Figure 8 eEF2 interaction with GAC elements

A and B - the structural model of the GAC elements with eEF2 (in red). The model derives from 80S·GMPPCP·mRNA·tRNA (PDB:6GQV) (Pellegrino et al., 2018) shown in figure 7 A. The right panel; individual ribosomal proteins are marked as follows: uL10 - hot pink, uL11 - olive, uL6 - wheat, eL40 - sand, uS12 – purple, and SRL - blue; B - the view as in A with rotation 180º around the Y axis. Inset on the left

- enlargement of the interface region of the P-stalk base consisting of the αββ motif of uL10 (amino acid region 126-154), the α-helix D of eEF2 domain I (amino acid region 172-188), and the β-sheet of domain G' (amino acid region 246-263); inset on the right - interaction of eEF2 and uS12. The α-helix A of domain III of eEF2 is shown in two conformations: yellow - pre-translational state (PDB:5JUO), gray - post-translational state (PDB:5JUU) (Abeyrathne, Koh, Grant, Grigorieff & Korostelev, 2016). The arrow represents the movement of α-helix by d 5 Å.

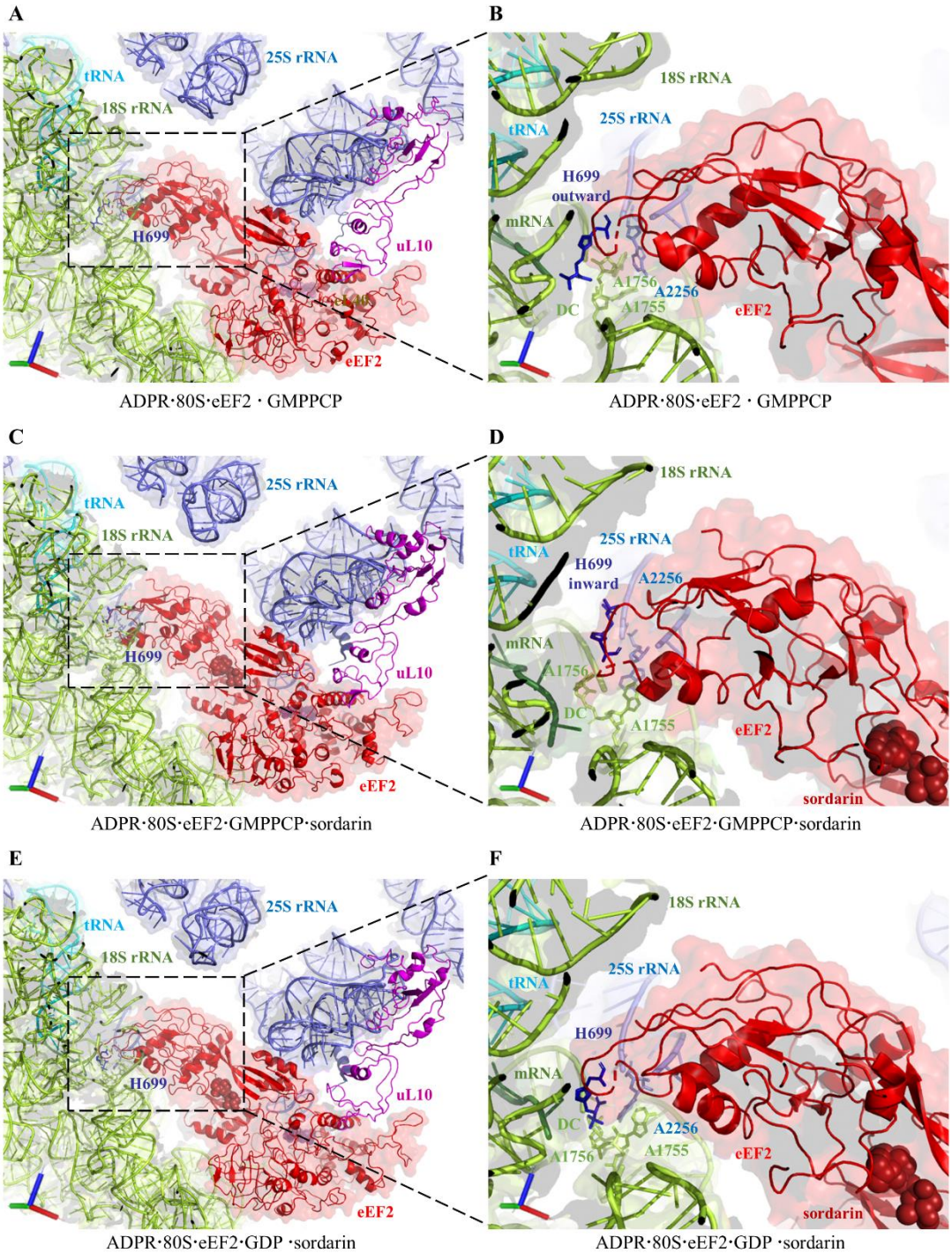

Figure 9 eEF2 interaction with the decoding center

A, C, E - overview of the interaction of domain IV of eEF2 with the decoding center within 80S in the presence of ADPR·80S·eEF2·GMPPCP (PDB:6GQV), ADPR·80S·eEF2·GMPPCP·sordarin (PDB:6GQ1), and ADPR·80S·eEF2·GDP·sordarin (PDB:6GQB) (Pellegrino et al., 2018). B, D, F - enlargement of the interaction region between domain IV of eEF2 and the decoding center focused on diphthamide modification in eEF2 at residue H699. uL10 - hot pink, 18S rRNA - lemon, 25S rRNA - slate, tRNA - cyan, mRNA - forest, H699 - blue, and eEF2 – red; sordarin in red as sphere representation. B - without sordarin binding, the H699 residue is outward to the decoding center (DC). D - with sordarin binding, H699 turns to an inward position. F - the H699 residue is in the intermediate state.

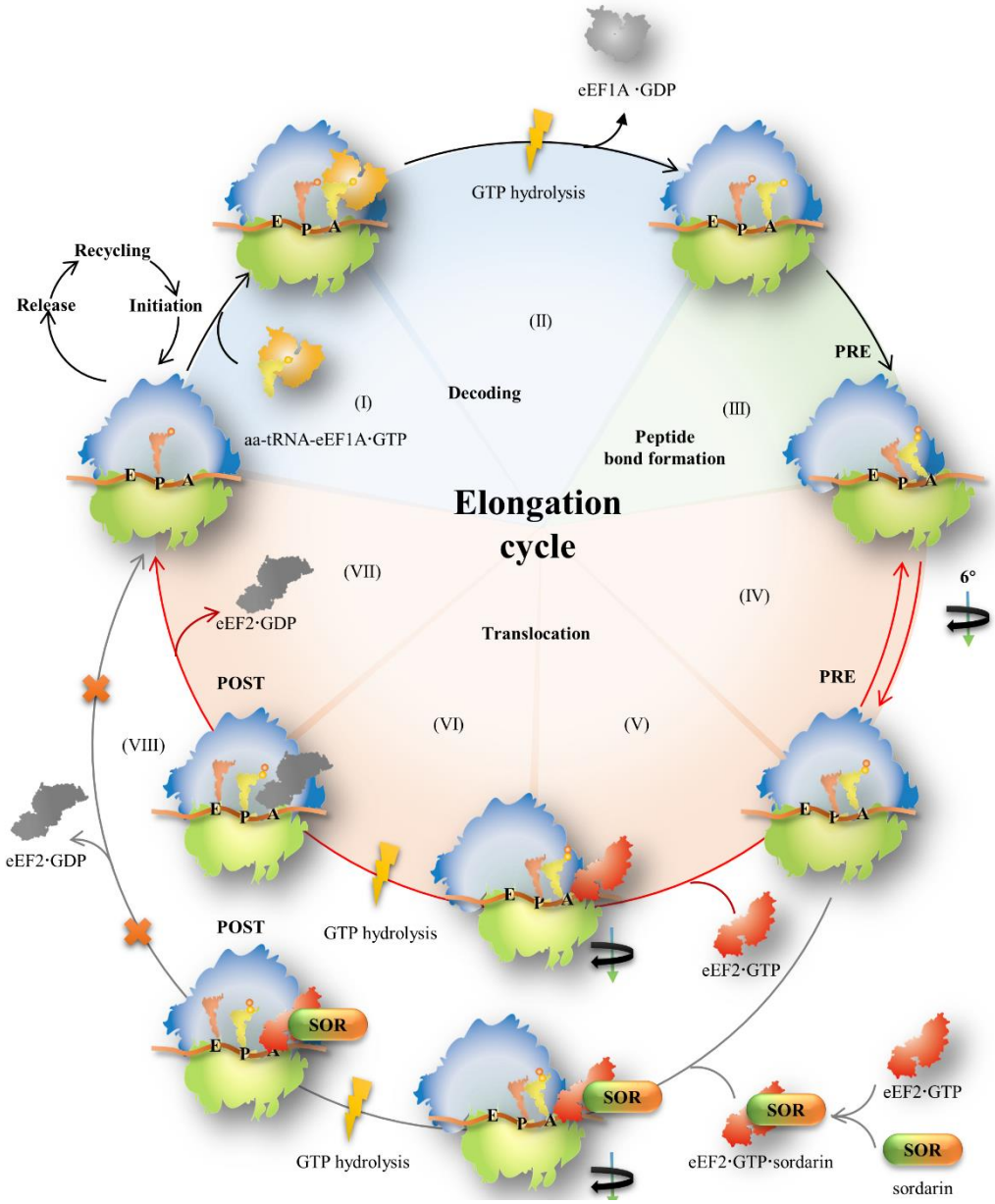

Figure 10 Model of the elongation step at the translational cycle with the proposed sordarin *modus operandi*

The translation process is composed of initiation, elongation cycle, termination, and recycling. The elongation cycle starts with the ribosome with the P site occupied by peptidyl-tRNA and an empty A site; the ternary complex eEF1A·GTP·aminoacyl-

tRNA delivers new aminoacyl-tRNA to the A site (I). During the decoding, proper aminoacyl-tRNA is accommodated triggering at the same time eEF1A-dependent GTP hydrolysis, allowing aminoacyl-tRNA to be fully accommodated into the A site; then, eEF1A·GDP leaves the ribosome (II). The aminoacyl-tRNA accommodation is followed by peptide bond formation (III). The nascent peptide chain is transferred to the A-site tRNA, leaving a deacylated tRNA in the P site, and with concomitant ribosome structure changes (III). All tRNAs are in a hybrid state with 40S ribosomal subunit rotation by 6° with peptidyl-tRNA in A/P and free tRNA in the P/E position (IV). During the translocation, the ribosome oscillates spontaneously between two states: pre-translocational state (rotated) and post-translocational state (unrotated) (IV). The mRNA shift by one codon exposing a new nucleotide triplet in the A site is catalyzed by trGTPase-eEF2, which recognizes and binds to 80S and stabilizes the rotated conformational state of the ribosome (V). This induces the head swivel of the 40S subunit, leading to the 'unlocking' of the 40S head-body interactions and accelerating the rate-limiting step of translocation: the movement of the tRNAs and mRNA on the small ribosomal subunit at the cost of GTP hydrolysis catalyzed by eEF2 (VI). This leads to exposition of the new codon in the A site to the ribosome with release of eEF2·GDP from the ribosomal complex (VII). The peptidyl-tRNA is located in the P site, and the E site is occupied by empty tRNA. The alternative pathway shows the sordarin action. Upon binding to eEF2, sordarin induces and provides stabilization forces for the extended conformation of eEF2 on the ribosome; the translocation step and GTP hydrolysis take place but the eEF2·sordarin complex stalls eEF2 on the ribosome and thus does not allow entering the 80S ribosome for the next round of elongation (VIII).

1967 **Tables**

1968 **Table 1 Sordarin analogs isolated from natural sources**

Strain	Compounds
Sordaria araneosa	Sordarin (Davoli, Engel, Werle, Sterner & Anke, 2002; Hauser & Sigg, 1971; Kudo, Matsuura, Hayashi, Fukushima & Eguchi, 2016; Tully et al., 2007), sordaricin (Weber, Meffert, Anke & Sterner, 2005) , hypoxysordarin (Daferner, Mensch, Anke & Sterner, 1999; Davoli, Engel, Werle, Sterner & Anke, 2002), hydroxysordarin (Davoli, Engel, Werle, Sterner & Anke, 2002; Weber, Meffert, Anke & Sterner, 2005)
Podospora pleiospora	Sordarin, sordaricin (Weber, Meffert, Anke & Sterner, 2005), hypoxysordarin 2(Davoli, Engel, Werle, Sterner & Anke, 2002; Weber, Meffert, Anke & Sterner, 2005)
Xylotumulus gibbisporus **YMJ863**	Sordarins C-F (Chang et al., 2014)
Hypoxylon croceum	Hypoxysordarin (Daferner, Mensch, Anke & Sterner, 1999; Davoli, Engel, Werle, Sterner & Anke, 2002)
Zopfielle marina **SANK21274**	Zofimarin (Chaichanan, Wiyakrutta, Pongtharangkul, Isarangkul & Meevootisom, 2014; Ogita, 1987; Tanaka, Moriguchi, Kizuka, Ono, Miyakoshi & Ogita, 2002; Vicente et al., 2009)
Xylaria **sp. Acra**	Zofimarin, isozofimarin (Chaichanan, Wiyakrutta, Pongtharangkul, Isarangkul & Meevootisom, 2014; Ogita, 1987; Tanaka, Moriguchi, Kizuka, Ono, Miyakoshi & Ogita, 2002; Vicente et al., 2009)
Xylaria **species *A19-91***	Xylarin a, b, c (Helaly, Thongbai & Stadler, 2018; Schneider, Anke & Sterner, 1995)
Graphium putredinis	GR 135402 (Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Kinsman et al., 1998)
Penicillium minioluteum	BE31405 (Okada et al., 1998)
unidentified fungus SCF1082A	SCH57404 (Coval, Puar, Phife, Terracciano & Patel, 1995)
Trichoderma harzianum **R5**	Trichosordarin A (Liang, Ma & Ji, 2020)
Morinia pestalozzioides	Moriniafungin (Basilio et al., 2006)
Curvularia hawaiiensis **TA26-15**	Moriniafungin (Basilio et al., 2006), moriniafungins B-G (Zhang et al., 2019)

Setosphaeria rostrata F3736	Moriniafungin(Park, Park, Kim, Lee & Kim, 2020)

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Table 2 Sordarin *in vitro* activity

Strains	Compounds	IC_{50} (μg/ml)	MIC (μg/ml)
Absidia corymbifera (Lichtheimia corymbifera)	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	4
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	16
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	4
Absidia glauca	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	20s-50s
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	10s-20s
Alternaria alternata (Alternaria rot fungus , Torula alternata)	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	>64
Alternaria porri	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
Aspergillus flavus	GM 193663(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	>64
	GM 211676(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	16-32
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.25-2

	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	4-62
	GR 135402(Kinsman et al., 1998)	-	125
Aspergillus flumigatus	sordarin(Kinsman et al., 1998)	-	>128
	FR290581(Hanadate et al., 2009)	-	128
	GM 193663(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	>64
	GM 211676(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	64
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	48
	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	≥64
	GR 135402(Kinsman et al., 1998)	-	>125
Aspergillus niger	sordarin(Okada et al., 1998)	-	>100
	BE-31405(Okada et al., 1998)	-	>100
Aspergillus ochraceus	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	10s
Blastoschizomyces capitatus	GM 237354(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	1-2
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.12
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.12

	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.12
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.12
Botrytis cinerea	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
Candida albicans	sordarin(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Okada et al., 1998; Schneider, Anke & Sterner, 1995)	0.01-0.4	3.13-100
	sordaricin(Hall et al., 2001; Weber, Meffert, Anke & Sterner, 2005)	0.036-0.1662	>125
	sordaricin B(Weber, Meffert, Anke & Sterner, 2005; Zhang et al., 2019)	-	8.4
	BE-31405(Okada et al., 1998)	-	3.13-50
	moriniafungin(Zhang et al., 2019)	0.9	2.6-6.25
	moriniafungin B(Zhang et al., 2019)	-	5.8
	moriniafungin C(Zhang et al., 2019)	-	7.6
	moriniafungin D(Zhang et al., 2019)	-	6.4
	moriniafungin E(Zhang et al., 2019)	-	2
	moriniafungin F(Zhang et al., 2019)	-	10.6
	moriniafungin G(Zhang et al., 2019)	-	9.4
	FR290581(Hanadate et al., 2009)	-	0.5
	R-135853(Kamai, Kakuta, Shibayama, Fukuoka & Kuwahara, 2005)	-	0.03
	GM 160575(Dominguez, Kelly, Kinsman, Marriott,	0.08	<0.001

	Gomez de las Heras & Martin, 1998)		
	GM 191519(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	0.005	0.12
	GM 193663(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	<0.005	0.03
	GM 211676(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	0.005	0.001
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)		0.001–0.03
	GM 237354(Aviles, Falcoz, San Roman & Gargallo-Viola, 2000; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)		0.001–0.03
	GR 135402(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Kinsman et al., 1998)	0.028-0.2	0.015-0.06
	GW 471552(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.008–0.06
	GW 471558(Chakraborty, Sejpal, Payghan, Ghoshal & Sengupta, 2016; Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001; Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.015–0.06
	GW 479821(Chakraborty,	-	0.001–0.002

Sejpal, Payghan, Ghoshal & Sengupta, 2016; Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)		
GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.002–0.015
GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.008–0.06
GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.002–0.015
2(Cuevas, Lavandera & Martos, 1999)	15	>207.4
6(Cuevas, Lavandera & Martos, 1999)	14	44.6
8(Cuevas, Lavandera & Martos, 1999)	32.8	186.1
10(Cuevas, Lavandera & Martos, 1999)	5.9	23.4
12(Cuevas, Lavandera & Martos, 1999)	74.4	185.9
15(Cuevas, Lavandera & Martos, 1999)	80.9	41.9
6-hydroxysordaricin(Hall et al., 2001)	>40	
7-hydroxysordarin(Hall et al., 2001)	0.08	>125
4’-O-demethylsordarin(Hall et al., 2001)	0.035	>125
2‘-O-acetylsordarin(Hall et al., 2001)	0.47	>125
sordarin-1-methyl ester(Hall et al., 2001)	>10	62
sordarin-1-glucose ester(Hall et al., 2001)	>10	>125
sordaricin-1-glucose ester(Hall et al., 2001)	>10	>125
sordarin-3-carboxylic acid(Hall et al., 2001)	>10	>125
3-deformyl-3-hydroxymethyl sordarin(Hall et al., 2001)	-	>31

	7-hydroxysordaricin(Hall et al., 2001)	>40	>125
	7-hydroxy-4-O-demethylsordarin(Hall et al., 2001)	0.04	>125
Candida glabrata	sordarin(Basilio et al., 2006; Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Okada et al., 1998)	0.2-8	50-125
	BE-31405(Okada et al., 1998)	-	0.78-12.5
	moriniafungin(Basilio et al., 2006)	1.8	25
	FR290581(Hanadate et al., 2009)	-	1
	R-135853(Kamai, Kakuta, Shibayama, Fukuoka & Kuwahara, 2005)	-	1
	GM 160575(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	0.4	>125
	GM 191519(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	0.5	31
	GM 193663(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	0.02	31
	GM 211676(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	0.01	8
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.03–0.5
	GM 237354(Herreros, Martinez, Almela, Marriott,	-	0.25–1

	De Las Heras & Gargallo-Viola, 1998)		
	GR 135402(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Kinsman et al., 1998)	0.8	0.03-125
	GW 471552(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	1–4
	GW 471558(Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001; Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.06–0.5
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.03–0.06
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.03–0.25
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.12–0.5
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.06–0.5
Candida guilliermondii	GW 471558(Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001)	-	>128.0
Candida kefyr (Kluyveromyces marxianus)	GM 193663(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.002–0.015
	GM 211676(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.004–0.015
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.001–0.008
	GM 237354(Herreros,	-	0.001–0.03

	Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)		
Candida krusei (Pichia kudriavzevii)	sordarin(Basilio et al., 2006; Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	>100	>100
	moriniafungin(Basilio et al., 2006)	21	>100
	GM 160575(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	>100	>125
	GM 191519(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	100	>125
	GM 193663(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	>100	>125
	GM 211676(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	100	>125
	GR 135402(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Kinsman et al., 1998)	>100	>125
	GW 471558(Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001; Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	128.0
Candida lusitaniae (Clavispora lusitaniae)	sordarin(Basilio et al., 2006)	>100	>100
	moriniafungin(Basilio et al., 2006)	70	100
	GW 471558(Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001)	-	>128.0
Candida neoformans	sordarin(Okada et al., 1998)	-	>128
	BE-31405(Okada et al., 1998)	-	6.25-100

	FR290581(Hanadate et al., 2009)	-	4
Candida parapsilosis	sordarin(Basilio et al., 2006; Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Okada et al., 1998)	>100	>125
	BE-31405(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Okada et al., 1998)	-	>100
	moriniafungin(Basilio et al., 2006)	39	100
	FR290581(Hanadate et al., 2009)	-	8
	GM 160575(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	>100	>125
	GM 191519(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	100	>125
	GM 193663(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	>100	>125
	GM 211676(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	100	>125
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	1–4
	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.25–16
	GR 135402(Kinsman et al.,	>100	>125

	1998)		
	GW 471552(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 471558(Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001; Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	128.0
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.5–2
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	2–4
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.5–4
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.25–1
Candida pseudotropicalis	GR 135402(Kinsman et al., 1998)	-	0.25
Candida tropicalis	FR290581(Hanadate et al., 2009)	-	0.5
	R-135853(Kamai, Kakuta, Shibayama, Fukuoka & Kuwahara, 2005)	-	0.5
	GM 193663(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.03–1
	GM 211676(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.015-0.5
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.008–0.12
	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-	-	0.002–0.12

	Viola, 1998)		
	GR 135402(Kinsman et al., 1998)	-	0.25
	GW 471552(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.03–0.12
	GW 471558(Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001; Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	≤0.0002–1.00
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.004–0.03
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.015–0.06
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.03–0.12
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.015–0.06
Cladosporium cladosporioides	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	<1
Colletotrichum gloeosporioides	moriniafungin(Park, Park, Kim, Lee & Kim, 2020)	-	1
Colletotrichum orbiculare	moriniafungin(Park, Park, Kim, Lee & Kim, 2020)	-	8
Cryptococcus neoformans (Filobasidiella neoformans)	sordarin(Basilio et al., 2006; Okada et al., 1998)	0.06-45	>100
	moriniafungin(Basilio et al., 2006; Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	19	100
	R-135853(Kamai, Kakuta,	-	0.5

	Shibayama, Fukuoka & Kuwahara, 2005)		
	GM 160575(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	0.01-100	0.25
	GM 191519(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998)	0.005	125
	GM 193663(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	0.2	2–8
	GM 211676(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	0.12	1–8
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.25–1
	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.015–0.25
	GR 135402(Dominguez, Kelly, Kinsman, Marriott, Gomez de las Heras & Martin, 1998; Kinsman et al., 1998)	0.2	0.25
Cunninghamella bertholletiae	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	2–4
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	16

	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	16
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	4
Curvularia lunata	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	>64
Endomyces ovetensis	sordarin(Okada et al., 1998)	-	>100
	BE-31405(Okada et al., 1998)	-	>100
Epidermophyton floccosum	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	2–32
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	16
Fusarium fujikuroi	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	xylarin a(Schneider, Anke & Sterner, 1995)	-	50s
	xylarin b(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin c(Schneider, Anke & Sterner, 1995)	-	>100

Fusarium oxysporum	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	xylarin a(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin b(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin c(Schneider, Anke & Sterner, 1995)	-	>100
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	8
Geotrichum clavatum	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	0.25–1
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.5
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.5
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.5
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	0.12
Microsporum canis (Arthroderma otae)	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	8
	GW 479821(Herreros,	-	>16

	Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)		
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	4
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	8
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	4
Microsporum gypseum (Arthroderma gypseum)	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	≥32
Mucor miehei	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	10s
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	1s
	xylarin a(Schneider, Anke & Sterner, 1995)	-	25s
	xylarin b(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin c(Schneider, Anke & Sterner, 1995)	-	>100
Nadsonia fulvescens	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
Nematospora coryli	sordarin(Daferner, Mensch, Anke & Sterner, 1999; Weber, Meffert, Anke & Sterner, 2005)	-	0.2
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999; Weber, Meffert, Anke & Sterner, 2005)	-	0.5
	xylarin a(Schneider, Anke & Sterner, 1995)	-	0.5
	xylarin b(Schneider, Anke & Sterner, 1995)	-	25s
	xylarin c(Schneider, Anke &	-	5s

	Sterner, 1995)		
Paecilomyces variotii	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	50s
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	2s
	xylarin a(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin b(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin c(Schneider, Anke & Sterner, 1995)	-	>100
Penicillium chrysogenum	sordarin(Okada et al., 1998)	-	>100
	BE-31405(Okada et al., 1998)	-	6.25-100
Penicillium islandicum	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	10s
	xylarin a(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin b(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin c(Schneider, Anke & Sterner, 1995)	-	>100
Penicillium notatum	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	2s
Pneumocystis carinii	GM 193663(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	<0.008	
	GM 211676(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	<0.008	
	GM 222712(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	<0.008	
	GM 237354(Herreros, Martinez, Almela, Marriott,	<0.008	

	De Las Heras & Gargallo-Viola, 1998)		
	GW 471552(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	0.001	
	GW 471558(Cuenca-Estrella, Mellado, Diaz-Guerra, Monzon & Rodriguez-Tudela, 2001)	<0.001	
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	8
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	4
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	8
Pseudallescheria boydii	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	<2
Rhizopus arrhizus (Rhizopus delemar)	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	2-4
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	2
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	2
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	4
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	1
Rhizopus oryzae	Moriniafungin (Park, Park, Kim, Lee & Kim, 2020)	-	0.125
Rhizopus stolonifer var.	Moriniafungin (Park, Park,	-	0.03125

stolonifer	Kim, Lee & Kim, 2020)		
Rhodotorula glutinis (Rhodosporidium toruloides)	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	xylarin a(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin b(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin c(Schneider, Anke & Sterner, 1995)	-	>100
Saccharomyce cerevisiae	sordarin(Basilio et al., 2006; Daferner, Mensch, Anke & Sterner, 1999; Davoli, Engel, Werle, Sterner & Anke, 2002; Okada et al., 1998; Tse, Balkovec, Blazey, Hsu, Nielsen & Schmatz, 1998)	0.15-3.9	1.56-50s
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999; Davoli, Engel, Werle, Sterner & Anke, 2002)	0.25-0.5	2s-50
	hypoxysordarin 2(Davoli, Engel, Werle, Sterner & Anke, 2002)	0.2-0.25	
	neosordarin(Davoli, Engel, Werle, Sterner & Anke, 2002)	0.2-0.3	
	xylarin a(Schneider, Anke & Sterner, 1995)	-	5-20
	xylarin b(Schneider, Anke & Sterner, 1995)	-	≥25
	xylarin c(Schneider, Anke & Sterner, 1995)	-	≥25s
	BE-31405(Okada et al., 1998)	-	3.13-50
	moriniafungin(Basilio et al., 2006)	1.2	10
	GR 135402(Kinsman et al., 1998)	-	0.13
Scedosporium apiospermum	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 515716(Herreros, Almela, Lozano, Gomez de las	-	>16

	Heras & Gargallo-Viola, 2001)		
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	8
Schizosaccharomyces pombe	sordarin(Okada et al., 1998)	-	≥100
	BE-31405(Okada et al., 1998)	-	0.78-6.25
Sporobolomyces roseus	sordarin(Weber, Meffert, Anke & Sterner, 2005)	-	1
	sordaricin(Weber, Meffert, Anke & Sterner, 2005)	-	25
	hypoxysordarin 1(Weber, Meffert, Anke & Sterner, 2005)	-	2.5
	hypoxysordarin 2(Weber, Meffert, Anke & Sterner, 2005)	-	>50
Trichophyton mentagrophytes	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	16-64
Trichophyton rubrum/Epidermophyton rubrum	GM 237354(Herreros, Martinez, Almela, Marriott, De Las Heras & Gargallo-Viola, 1998)	-	≥64
	GW 479821(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	>16
	GW 515716(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	8
	GW 570009(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	16
	GW 587270(Herreros, Almela, Lozano, Gomez de las Heras & Gargallo-Viola, 2001)	-	8
Trichophyton verrucosum	sordaricin B(Weber, Meffert, Anke & Sterner, 2005; Zhang et al., 2019)	-	>64
Trichosporon beigelii	GM 237354(Herreros, Martinez, Almela, Marriott,	-	<4

	De Las Heras & Gargallo-Viola, 1998)		
Trichosporon cutaneum	sordarin(Okada et al., 1998)	-	>100
	BE-31405(Okada et al., 1998)	-	>100
Ustilago nuda	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	>50
	xylarin a(Schneider, Anke & Sterner, 1995)	-	25s
	xylarin b(Schneider, Anke & Sterner, 1995)	-	>100
	xylarin c(Schneider, Anke & Sterner, 1995)	-	>100
Zygorhynchus moelleri	sordarin(Daferner, Mensch, Anke & Sterner, 1999)	-	20s
	hypoxysordarin 1(Daferner, Mensch, Anke & Sterner, 1999)	-	20s

The data presented are provided in the range of inhibition; s: fungistatic, the growth restarted after removal of the compound.

-: not determined

1976 **Table 3 *In vivo* activity of sordarins toward *Candida albicans* infections**

analogs	model	dose (mg/kg)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC(μg·h/ml)	V_{ss} (L/kg)
Sordarin(Hanadate et al., 2009)	mouse	2	0.02	0.33	-	-
FR290581(Hanadate et al., 2009)	mouse	2	1	3.4	-	-
R-135853(Weber, Meffert, Anke & Sterner, 2005)	mouse, intravenous	2	-	0.47	0.509	-
	mouse, oral	20	2.32	1.1	3.19	-
GM 237354(Aviles, Falcoz, San Roman & Gargallo-Viola, 2000; Aviles, Pateman, San Roman, Guillen, Gomez De Las Heras & Gargallo-Viola, 2001; Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000)	mouse, intravenously	5	3.16	0.36	2.33	-
	mouse, intravenously	40	21.8	0.4	30.7	-
	mouse, intravenously	50	23.04	0.52	46.04	-
	mouse	50	23	0.85	46	-
	rat	10	7.2	0.8	11.8	-
	mouse	20	33.6	0.28	17.8	0.39
	rat	20	33.1	0.59	38.1	0.44
	rabbit	20	89.1	0.3	42.4	0.23
	monkey	20	72.4	1.73	161	0.31
GM 222712(Aviles, Pateman, San Roman, Guillen, Gomez De Las Heras & Gargallo-Viola, 2001)	mouse	20	22.3	0.2	9	0.6
	monkey	20	102.9	3.03	348	0.25
GM 193633(Aviles, Pateman, San Roman, Guillen, Gomez De Las Heras & Gargallo-Viola, 2001; Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000)	mouse	50	51.8	0.8	79.5	-
	rat	10	6.6	0.7	8.5	-
	mouse	20	38.1	0.45	24.3	0.53
	monkey	20	69.3	1.75	180	0.28
	rat	20	45.4	0.51	33.7	0.44
	rat	10	16.8	0.55	13.3	0.6
GW 471552(Martinez et al., 2001)	rat	10	-	-	-	-
GW 471558(Gargallo-	mouse	20	-	0.6	27.9	0.55
	rat	10	-	0.75	14.7	0.7
	dog	1	-	0.28	1.34	0.26

Viola, 1999; Odds, 2001)						
GW	mouse	20	-	0.44	25.9	0.49
531920(Gargallo-	rat	1	-	1.45	2.1	0.7
Viola, 1999; Odds, 2001)	dog	1	-	0.42	3.7	0.2
azasordarin(Serrano-Wu et al., 2003)	mouse, oral	20	-	-	-	0.49
7a(Serrano-Wu et al., 2003)	mouse, oral	20	5.946	2.1	-	7.1
7b(Serrano-Wu et al., 2003)	mouse, oral	20	3.882	3.1	-	1.6

-: not determined; dose (mg/kg) – intravenous dose of administration, C_{max} (μg/mL) - maximum concentration of drug in serum, $T_{1/2}$ (h) - half-life, AUC(μg·h/ml) – the area under the concentration-time curve, V_{SS} (L/kg) - the volume of distribution at steady state

Table 4 Sordarin *in vivo* activity to *Pneumocystis carinii*

sordarins	model	dose (mg/kg)	log cysts/g of lung	reduction (%)
control(Jimenez, Martinez, Aliouat el, Caballero, Dei-Cas & Gargallo-Viola, 2002; Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000)	Wistar rats	-	6.9 ± 0.4	-
	nude rats	-	7.3 ± 0.2	-
	Female Wistar rats	-	7.6 ± 0.2	-
Septrin(Jimenez, Martinez, Aliouat el, Caballero, Dei-Cas & Gargallo-Viola, 2002)	Wistar rats	50/250	4.9 ± 0.4	98.96
	nude rats	50/250	6.7 ± 0.2	80.04
GW 471552(Jimenez, Martinez, Aliouat el, Caballero, Dei-Cas & Gargallo-Viola, 2002)	Wistar rats	1	5.0 ± 0.6	98.21
		5	5.1 ± 0.2	98.88
	nude rats	0.25	5.0 ± 0.8	99.49
		0.5	3.2 ± 0.2	99.99
GW 471558(Jimenez, Martinez, Aliouat el, Caballero, Dei-Cas & Gargallo-Viola, 2002)	Wistar rats	1	5.0 ± 0.6	97.9
		5	4.9 ± 0.4	98.96
	nude rats	0.25	6.6 ± 0.4	74.88
		0.5	<3	>99.99
GM 19366(Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000)	Female Wistar rats	0.1	6.7 ± 0.9	89.81
		1	4.7 ± 0.2	99.9
		5	4.8 ± 0.3	99.86
GM 237354(Martinez, Aviles, Jimenez, Caballero & Gargallo-Viola, 2000)	Female Wistar rats	0.1	5.8 ± 0.9	99.82
		1	4.6 ± 0.1	99.98
		5	3.4 ± 0.2	99.99

dose (mg/kg) – intravenous dose of administration, log cysts/g of lung – the mean (± standard deviation) log number of cysts, reduction (%) – the reduction in the number of cysts in the lungs of treated versus untreated animals.

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Table 5 eEF2 mutations conferring resistance to sordarin determined by genetic analyses

eEF2 domain	Mutation	Sordarins	S/R	Mutation IC_{50} (μg/ml)	Control IC_{50} (μg/ml)
I	R180G	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	15	0.5-1
	V187F	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	20	0.5-1
III	Q490E	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	45	0.5-1
	C517A	Sordarin(Shastry et al., 2001)	S	0.02	0.5
	C517M	Sordarin(Shastry et al., 2001)	S	0.048	0.5
	V518A	Sordarin(Shastry et al., 2001)	S	0.12	0.5
	L519A	Sordarin(Shastry et al., 2001)	S	0.046	0.5
	L519K	Sordarin(Shastry et al., 2001)	R	0.6	0.5
	L519Q	Sordarin(Shastry et al., 2001)	S	0.05	0.5
	T520A	Sordarin(Shastry et al., 2001)	S	0.046	0.5
	T520C	Sordarin(Shastry et al., 2001)	S	0.11	0.5
	Y521A	Sordarin(Shastry et al., 2001)	R	7.8	0.5
	Y521D	Sordarin(Capa, Mendoza, Lavandera, Gomez de las Heras & Garcia-Bustos, 1998; Justice et al., 1998)	R	60	0.5-1
	Y521I	Sordarin(Shastry et al., 2001)	R	0.65	0.5
	Y521N	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	20	0.5-1
	Y521Q	Sordarin(Shastry et al., 2001)	R	3.5	0.5
	Y521S	Sordarin(Justice et al., 1998)	R	35	0.5-1
				12.0	0.5
	Y521W	Sordarin(Shastry et al., 2001)	S	0.2	0.5
	M522A	Sordarin(Shastry et al., 2001)	S	0.34	0.5
	M522I	Sordarin(Shastry et al., 2001)	S	0.045	0.5
	S523A	Sordarin(Shastry et al., 2001)	R	3.0	0.5
	S523E	Sordarin(Shastry et al., 2001)	R	>100	0.5
	S523F	Sordarin(Harger, Meskauskas, Nielsen, Justice	R	>100	0.5-1

		& Dinman, 2001; Justice et al., 1998)			
	S523G	Sordarin(Shastry et al., 2001)	R	8.0	0.5
	S523N	Sordarin(Shastry et al., 2001)	R	75.0	0.5
	S523P	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	>100	0.5-1
	E524A	Sordarin(Shastry et al., 2001)	S	0.05	0.5
	E524D	Sordarin(Shastry et al., 2001)	S	0.044	0.5
	E524P	Sordarin(Shastry et al., 2001)	R	>100	0.5
	S525A	Sordarin(Shastry et al., 2001)	R	0.04	0.5
	I529T^	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	30	0.5-1
IV	P559L	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	>100	0.5-1
	P559R	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	>100	0.5-1
	A562P	Sordarin(Capa, Mendoza, Lavandera, Gomez de las Heras & Garcia-Bustos, 1998; Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	>100	0.5-1
V	P727S	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	>100	0.5-1
	V774F	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	>100	0.5-1
	G790Δ	Sordarin(Harger, Meskauskas, Nielsen, Justice & Dinman, 2001; Justice et al., 1998)	R	>100	0.5-1

Abbreviations and symbols: Δ, deletion; S, sensitivity; R, resistance; mutation IC_{50} (μg/ml), half maximal inhibitory concentration of the mutants treated by sordarin;

control IC_{50} (μg/ml), half maximal inhibitory concentration of the mutants treated by sordarin.

1994 **Table 6 P-protein mutations in relation to sordarin acion**

P-proteins	Mutation	Sordarins	S/R	Mutation IC_{50} (μg/ml)	Control IC_{50} (μg/ml)
uL10	A117E	GM193663(Santos & Ballesta, 2002)	R	ND	
	P122R	GM193663(Santos & Ballesta, 2002)	R	ND	
	G124V	GM193663(Santos & Ballesta, 2002)	R	ND	
	S134Δ	Sordarin(Justice, Ku, Hsu, Carniol, Schmatz & Nielsen, 1999)	R	20	0.5
	Q137P	Sordarin(Justice, Ku, Hsu, Carniol, Schmatz & Nielsen, 1999)	R	ND	
	Q137K	Sordarin(Justice, Ku, Hsu, Carniol, Schmatz & Nielsen, 1999)	R	30	0.5
	Q139H	GM193663(Gomez-Lorenzo & Garcia-Bustos, 1998)	R	1.36-1.12	0.01
	T143L	Sordarin(Justice, Ku, Hsu, Carniol, Schmatz & Nielsen, 1999)	R	ND	
	T143A	Sordarin(Justice, Ku, Hsu, Carniol, Schmatz & Nielsen, 1999)	R	30	0.5
	T144A	GM193663(Gomez-Lorenzo & Garcia-Bustos, 1998)	R	5.83-16.72	0.01
P1A	ΔP1A	GM193663(Gomez-Lorenzo & Garcia-Bustos, 1998)	S	1.16	16.72
P1B	ΔP1B	GM193663(Gomez-Lorenzo & Garcia-Bustos, 1998)	S	12.75	16.72
P2A	ΔP2A	GM193663(Gomez-Lorenzo & Garcia-Bustos, 1998)	S	14.56	16.72

P2B	ΔP2B	GM193663(Gomez-Lorenzo & Garcia-Bustos, 1998)	S	1.22	16.72
P1A-P2B	ΔP1A, P2B	GM193663(Gomez-Lorenzo & Garcia-Bustos, 1998)	S	0.25	16.72
P1B-P2A	ΔP1B, P2A	GM193663(Gomez-Lorenzo & Garcia-Bustos, 1998)	S	12.50	16.72

Abbreviations and symbols: Δ, deletion; ND, not described; S, sensitivity; R, resistance, mutation IC_{50} (μg/ml), half maximal inhibitory concentration of the mutants treated by sordarin; control IC_{50} (μg/ml), half maximal inhibitory concentration of the mutants treated by sordarin